10,019,243

(19) Eur päisches Patentamt
Eur pean Pat nt Offic
Office européen des brevets

(11) Publication number: 0 612 846 A1

(12) EUROPEAN PATENT APPLICATION

(21) Application number: 94101207.2

(22) Date of filing: 27.01.94

(83) Declaration under Rule 28(4) EPC (expert solution)

(51) Int. Cl.5: C12N 15/27, C07K 3/00, C12P 21/02, C07K 13/00, G06F 15/60

(30) Priority: 28.01.93 US 10099

(43) Date of publication of application: 31.08.94 Bulletin 94/35

(84) Designated Contracting States: AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE

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(54) G-CSF analog compositions and methods.

(57) Provided herein are granulocyte colony stimulating factor ("G-CSF") analogs, compositions containing such analogs, and related compositions. In another aspect, provided herein are nucleic acids encoding the present analogs or related nucleic acids, related host cells and vectors. In yet another aspect, provided herein are computer programs and apparatuses for expressing the three dimensional structure of G-CSF and analogs thereof. In another aspect, provided herein are methods for rationally designing G-CSF analogs and related compositions. In yet another aspect, provided herein are methods for treatment using the present G-CSF analogs.

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Field of the Invention

This invention relates to granulocyte colony stimulating factor ("G-CSF") analogs, compositions containing such analogs, and related compositions. In another aspect, the present invention relates to nucleic acids encoding the present analogs or related nucleic acids, related host cells and vectors. In another aspect, the invention relates to computer programs and apparatuses for expressing the three dimensional structure of G-CSF and analogs thereof. In another aspect, the invention relates to methods for rationally designing G-CSF analogs and related compositions. In yet another aspect, the present invention relates to methods for treatment using the present G-CSF analogs.

Background

Hematopoiesis is controlled by two systems: the cells within the bone marrow microenvironment and growth factors. The growth factors, also called colony stimulating factors, stimulate committed progenitor cells to proliferate and to form colonies of differentiating blood cells. One of these factors is granulocyte colony stimulating factor, herein called G-CSF, which preferentially stimulates the growth and development of neutrophils, indicating a potential use in neutropenic states. Welte et al., PNAS-USA 82: 1526-1530 (1985); Souza et al., Science 232: 61-65 (1986) and Gabrilove, J. Seminars in Hematology 26: (2) 1-14 (1989).

In humans, endogenous G-CSF is detectable in blood plasma. Jones et al., Bailliere's Clinical Hematology 2 (1): 83-111 (1988). G-CSF is produced by fibroblasts, macrophages, T cells trophoblasts, endothelial cells and epithelial cells and is the expression product of a single copy gene comprised of four exons and five introns located on chromosome seventeen. Transcription of this locus produces a mRNA species which is differentially processed, resulting in two forms of G-CSF mRNA, one version coding for a protein of 177 amino acids, the other coding for a protein of 174 amino acids, Nagata et al., EMBO J 5: 575-581 (1986), and the form comprised of 174 amino acids has been found to have the greatest specific in vivo biological activity. G-CSF is species cross-reactive, such that when human G-CSF is administered to another mammal such as a mouse, canine or monkey, sustained neutrophil leukocytosis is elicited. Moore et al., PNAS-USA 84: 7134-7138 (1987).

Human G-CSF can be obtained and purified from a number of sources. Natural human G-CSF (nhG-CSF) can be isolated from the supernatants of cultured human tumor cell lines. The development of recombinant DNA technology, see, for instance, U.S. Patent 4,810,643 (Souza) incorporated herein by reference, has enabled the production of commercial scale quantities of G-CSF in glycosylated form as a product of eukaryotic host cell expression, and of G-CSF in non-glycosylated form as a product of prokaryotic host cell expression.

G-CSF has been found to be useful in the treatment of indications where an increase in neutrophils will provide benefits. For example, for cancer patients, G-CSF is beneficial as a means of selectively stimulating neutrophil production to compensate for hematopoietic deficits resulting from chemotherapy or radiation therapy. Other indications include treatment of various infectious diseases and related conditions, such as sepsis, which is typically caused by a metabolite of bacteria. G-CSF is also useful alone, or in combination with other compounds, such as other cytokines, for growth or expansion of cells in culture, for example, for bone marrow transplants.

Signal transduction, the way in which G-CSF effects cellular metabolism, is not currently thoroughly understood. G-CSF binds to a cell-surface receptor which apparently initiates the changes within particular progenitor cells, leading to cell differentiation.

Various altered G-CSF's have been reported. Generally, for design of drugs, certain changes are known to have certain structural effects. For example, deleting one cysteine could result in the unfolding of a molecule which is, in its unaltered state, is normally folded via a disulfide bridge. There are other known methods for adding, deleting or substituting amino acids in order to change the function of a protein.

Recombinant human G-CSF mutants have been prepared, but the method of preparation does not include overall structure/function relationship information. For example, the mutation and biochemical modification of Cys 18 has been reported. Kuga et al., Biochem. Biophy. Res. Comm 159: 103-111 (1989); Lu et al., Arch. Biochem. Biophys. 268: 81-92 (1989).

In U.S. Patent No. 4, 810, 643, entitled, "Production of Pluripotent Granulocyte Colony-Stimulating Factor" (as cited above), polypeptide analogs and peptide fragments of G-CSF are disclosed generally. Specific G-CSF analogs disclosed include those with the cysteins at positions 17, 36, 42, 64, and 74 (of the 174 amino acid species or of those having 175 amino acids, the additional amino acid being an N-terminal methionine) substituted with another amino acid, (such as serine), and G-CSF with an alanine in the first (N-

terminal) position.

EP 0 335 423 entitled "Modified human G-CSF" reportedly discloses the modification of at least one amino group in a polypeptide having hG-CSF activity.

EP 0 272 703 entitled "Novel Polypeptide" reportedly discloses G-CSF derivatives having an amino acid substituted or deleted at or "in the neighborhood" of the N terminus.

EP 0 459 630, entitled "Polypeptides" reportedly discloses derivatives of naturally occurring G-CSF having at least one of the biological properties of naturally occurring G-CSF and a solution stability of at least 35% at 5 mg/ml in which the derivative has at least Cys^{17} of the native sequence replaced by a Ser^{17} residue and Asp^{27} of the native sequence replaced by a Ser^{27} residue.

EP 0 256 843 entitled "Expression of G-CSF and Muteins Thereof and Their Uses" reportedly discloses a modified DNA sequence encoding G-CSF wherein the N-terminus is modified for enhanced expression of protein in recombinant host cells, without changing the amino acid sequence of the protein.

EP 0 243 153 entitled "Human G-CSF Protein Expression" reportedly discloses G-CSF to be modified by inactivating at least one yeast KEX2 protease processing site for increased yield in recombinant production using yeast.

Shaw, U.S. Patent No. 4,904,584, entitled "Site-Specific Homogeneous Modification of Polypeptides," reportedly discloses lysine altered proteins.

WO/9012874 reportedly discloses cysteine altered variants of proteins.

Australian patent application Document No. AU-A-10948/92, entitled, "Improved Activation of Recombinant Proteins" reportedly discloses the addition of amino acids to either terminus of a G-CSF molecule for the purpose of aiding in the folding of the molecule after prokaryotic expression.

Australian patent application Document No. AU-A-76380/91, entitled, "Muteins of the Granulocyte Colony Stimulating Factor (G-CSF)" reportedly discloses muteins of the granulocyte stimulating factor G-CSF in the sequence Leu-Gly-His-Ser-Leu-Gly-Ile at position 50-56 of G-CSF with 174 amino acids, and position 53 to 59 of the G-CSF with 177 amino acids, or/and at least one of the four histadine residues at positions 43, 79, 156 and 170 of the mature G-CSF with 174 amino acids or at positions 46, 82, 159, or 173 of the mature G-CSF with 177 amino acids.

GB 2 213 821, entitled "Synthetic Human Granulocyte Colony Stimulating Factor Gene" reportedly discloses a synthetic G-CSF-encoding nucleic acid sequence incorporating restriction sites to facilitate the cassette mutagenesis of selected regions, and flanking restriction sites to facilitate the incorporation of the gene into a desired expression system.

G-CSF has reportedly been crystallized to some extent, e.g., EP 344 796, and the overall structure of G-CSF has been surmised, but only on a gross level. Bazan, Immunology Today 11: 350-354 (1990); Parry et al., J. Molecular Recognition 8: 107-110 (1988). To date, there have been no reports of the overall structure of G-CSF, and no systematic studies of the relationship of the overall structure and function of the molecule, studies which are essential to the systematic design of G-CSF analogs. Accordingly, there exists a need for a method of this systematic design of G-CSF analogs, and the resultant compositions.

Summary of the Invention

The three dimensional structure of G-CSF has now been determined to the atomic level. From this three-dimensional structure, one can now forecast with substantial certainty how changes in the composition of a G-CSF molecule may result in structural changes. These structural characteristics may be correlated with biological activity to design and produce G-CSF analogs.

Although others had speculated regarding the three dimensional structure of G-CSF, Bazan, Immunology Today 11: 350-354 (1990); Parry et al., J. Molecular Recognition 8: 107-110 (1988), these speculations were of no help to those wishing to prepare G-CSF analogs either because the surmised structure was incorrect (Parry et al., supra) and/or because the surmised structure provided no detail correlating the constituent moieties with structure. The present determination of the three-dimensional structure to the atomic level is by far the most complete analysis to date, and provides important information to those wishing to design and prepare G-CSF analogs. For example, from the present three dimensional structural analysis, precise areas of hydrophobicity and hydrophilicity have been determined.

Relative hydrophobicity is important because it directly relates to the stability of the molecule. Generally, biological molecules, found in aqueous environments, are externally hydrophilic and internally hydrophobic; in accordance with the second law of thermodynamics provides, this is the lowest energy state and provides for stability. Although one could have speculated that G-CSF's internal core would be hydrophobic, and the outer areas would be hydrophilic, one would have had no way of knowing specific hydrophobic or hydrophilic areas. With the presently provided knowledge of areas of hydrophobic-

ity/philicity, one may forecast with substantial certainty which changes to the G-CSF molecule will affect the overall structure of the molecule.

As a general rule, one may use knowledge of the geography of the hydrophobic and hydrophilic regions to design analogs in which the overall G-CSF structure is not changed, but change does affect biological activity ("biological activity" being used here in its broadest sense to denote function). One may correlate biological activity to structure. If the structure is not changed, and the mutation has no effect on biological activity, then the mutation has no biological function. If, however, the structure is not changed and the mutation does affect biological activity, then the residue (or atom) is essential to at least one biological function. Some of the present working examples were designed to provide no change in overall structure, yet have a change in biological function.

Based on the correlation of structure to biological activity, one aspect of the present invention relates to G-CSF analogs. These analogs are molecules which have more, fewer, different or modified amino acid residues from the G-CSF amino acid sequence. The modifications may be by addition, substitution, or deletion of one or more amino acid residues. The modification may include the addition or substitution of analogs of the amino acids themselves, such as peptidomimetics or amino acids with altered moieties such as altered side groups. The G-CSF used as a basis for comparison may be of human, animal or recombinant nucleic acid-technology origin (although the working examples disclosed herein are based on the recombinant production of the 174 amino acid species of human G-CSF, having an extra N-terminus methionyl residue). The analogs may possess functions different from natural human G-CSF molecule, or may exhibit the same functions, or varying degrees of the same functions. For example, the analogs may be designed to have a higher or lower biological activity, have a longer shelf-life or a decrease in stability, be easier to formulate, or more difficult to combine with other ingredients. The analogs may have no hematopoietic activity, and may therefore be useful as an antagonist against G-CSF effect (as, for example, in the overproduction of G-CSF). From time to time herein the present analogs are referred to as proteins or peptides for convenience, but contemplated herein are other types of molecules, such as peptidomimetics or chemically modified peptides.

In another aspect, the present invention relates to related compositions containing a G-CSF analog as an active ingredient. The term, "related composition," as used herein, is meant to denote a composition which may be obtained once the identity of the G-CSF analog is ascertained (such as a G-CSF analog labeled with a detectable label, related receptor or pharmaceutical composition). Also considered a related composition are chemically modified versions of the G-CSF analog, such as those having attached at least one polyethylene glycol molecule.

For example, one may prepare a G-CSF analog to which a detectable label is attached, such as a fluorescent, chemiluminescent or radioactive molecule.

Another example is a pharmaceutical composition which may be formulated by known techniques using known materials, see, e.g., Remington's Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing Co., Easton, Pennsylvania 18042) pages 1435-1712, which are herein incorporated by reference. Generally, the formulation will depend on a variety of factors such as administration, stability, production concerns and other factors. The G-CSF analog may be administered by injection or by pulmonary administration via inhalation. Enteric dosage forms may also be available for the present G-CSF analog compositions, and therefore oral administration may be effective. G-CSF analogs may be inserted into liposomes or other microcarriers for delivery, and may be formulated in gels or other compositions for sustained release. Although preferred compositions will vary depending on the use to which the composition will be put, generally, for G-CSF analogs having at least one of the biological activities of natural G-CSF, preferred pharmaceutical compositions are those prepared for subcutaneous injection or for pulmonary administration via inhalation, although the particular formulations for each type of administration will depend on the characteristics of the analog.

Another example of related composition is a receptor for the present analog. As used herein, the term "receptor" indicates a moiety which selectively binds to the present analog molecule. For example, antibodies, or fragments thereof, or "recombinant antibodies" (see Huse et al., Science 246:1275 (1989)) may be used as receptors. Selective binding does not mean only specific binding (although binding-specific receptors are encompassed herein), but rather that the binding is not a random event. Receptors may be on the cell surface or intra- or extra-cellular, and may act to effectuate, inhibit or localize the biological activity of the present analogs. Receptor binding may also be a triggering mechanism for a cascade of activity indirectly related to the analog itself. Also contemplated herein are nucleic acids, vectors containing such nucleic acids and host cells containing such nucleic acids which encode such receptors.

Another example of a related composition is a G-CSF analog with a chemical moiety attached. Generally, chemical modification may alter biological activity or antigenicity of a protein, or may alter other

characteristics, and these factors will be taken into account by a skilled practitioner. As noted above, one example of such chemical moiety is polyethylene glycol. Modification may include the addition of one or more hydrophilic or hydrophobic polymer molecules, fatty acid molecules, or polysaccharide molecules. Examples of chemical modifiers include polyethylene glycol, alkilpolyethylene glycols, DI-poly(amino acids), polyvinylpyrrolidone, polyvinyl alcohol, pyran copolymer, acetic acid/acylation, proprionic acid, palmitic acid, stearic acid, dextran, carboxymethyl cellulose, pullulan, or agarose. See, Francis, *Focus on Growth Factors* 3: 4-10 (May 1992) (published by Mediscript, Mountview Court, Friern Barnet Lane, London N20 OLD, UK). Also, chemical modification may include an additional protein or portion thereof, use of a cytotoxic agent, or an antibody. The chemical modification may also include lecithin.

In another aspect, the present invention relates to nucleic acids encoding such analogs. The nucleic acids may be DNAs or RNAs or derivatives thereof, and will typically be cloned and expressed on a vector, such as a phage or plasmid containing appropriate regulatory sequences. The nucleic acids may be labeled (such as using a radioactive, chemiluminescent, or fluorescent label) for diagnostic or prognostic purposes, for example. The nucleic acid sequence may be optimized for expression, such as including codons preferred for bacterial expression. The nucleic acid and its complementary strand, and modifications thereof which do not prevent encoooding of the desired analog are here contemplated.

In another aspect, the present invention relates to host cells containing the above nucleic acids encoding the present analogs. Host cells may be eukaryotic or prokaryotic, and expression systems may include extra steps relating to the attachment (or prevention) of sugar groups (glycosylation), proper folding of the molecule, the addition or deletion of leader sequences or other factors incident to recombinant expression.

In another aspect the present invention relates to antisense nucleic acids which act to prevent or modify the type or amount of expression of such nucleic acid sequences. These may be prepared by known methods.

In another aspect of the present invention, the nucleic acids encoding a present analog may be used for gene therapy purposes, for example, by placing a vector containing the analog-encoding sequence into a recipient so the nucleic acid itself is expressed inside the recipient who is in need of the analog composition. The vector may first be placed in a carrier, such as a cell, and then the carrier placed into the recipient. Such expression may be localized or systemic. Other carriers include non-naturally occurring carriers, such as liposomes or other microcarriers or particles, which may act to mediate gene transfer into a recipient.

The present invention also provides for computer programs for the expression (such as visual display) of the G-CSF or analog three dimensional structure, and further, a computer program which expresses the identity of each constituent of a G-CSF molecule and the precise location within the overall structure of that constituent, down to the atomic level. Set forth below is one example of such program. There are many currently available computer programs for the expression of the three dimensional structure of a molecule. Generally, these programs provide for inputting of the coordinates for the three dimensional structure of a molecule (i.e., for example, a numerical assignment for each atom of a G-CSF molecule along an x, y, and z axis), means to express (such as visually display) such coordinates, means to alter such coordinates and means to express an image of a molecule having such altered coordinates. One may program crystallographic information, i.e., the coordinates of the location of the atoms of a G-CSF molecule in three dimension space, wherein such coordinates have been obtained from crystallographic analysis of said G-CSF molecule, into such programs to generate a computer program for the expression (such as visual display) of the G-CSF three dimensional structure. Also provided, therefore, is a computer program for the expression of G-CSF analog three dimensional structure. Preferred is the computer program Insight II, version 4, available from Biosym, San Diego, California, with the coordinates as set forth in FIGURE 5 input. Preferred expression means is on a Silicon Graphics 320 VGX computer, with Crystal Eyes glasses (also available from Silicon Graphics), which allows one to view the G-CSF molecule or its analog stereoscopically. Alternatively, the present G-CSF crystallographic coordinates and diffraction data are also deposited in the Protein Data Bank, Chemistry Department, Brookhaven National Laboratory, Upton, New York 119723, USA. One may use these data in preparing a different computer program for expression of the three dimensional structure of a G-CSF molecule or analog thereof. Therefore, another aspect of the present invention is a computer program for the expression of the three dimensional structure of a G-CSF molecule. Also provided is said computer program for visual display of the three dimensional structure of a G-CSF molecule; and further, said program having means for altering such visual display. Apparatus useful for expression of such computer program, particularly for the visual display of the computer image of said three dimensional structure of a G-CSF molecule or analog thereof is also therefore here provided, as well as means for preparing said computer program and apparatus.

The computer program is useful for preparation of G-CSF analogs because one may select specific sites on the G-CSF molecule for alteration and readily ascertain the effect the alteration will have on the overall structure of the G-CSF molecule. Selection of said site for alteration will depend on the desired biological characteristic of the G-CSF analog. If one were to randomly change said G-CSF molecule (r-met-hu-G-CSF) there would be 175^{20} possible substitutions, and even more analogs having multiple changes, additions or deletions. By viewing the three dimensional structure wherein said structure is correlated with the composition of the molecule, the selection for sites of alteration is no longer a random event, but sites for alteration may be determined rationally.

As set forth above, identity of the three dimensional structure of G-CSF, including the placement of each constituent down to the atomic level has now yielded information regarding which moieties are necessary to maintain the overall structure of the G-CSF molecule. One may therefore select whether to maintain the overall structure of the G-CSF molecule when preparing a G-CSF analog of the present invention, or whether (and how) to change the overall structure of the G-CSF molecule when preparing a G-CSF analog of the present invention. Optionally, once one has prepared such analog, one may test such analog for a desired characteristic.

One may, for example, seek to maintain the overall structure possessed by a non-altered natural or recombinant G-CSF molecule. The overall structure is presented in Figures 2, 3, and 4, and is described in more detail below. Maintenance of the overall structure may ensure receptor binding, a necessary characteristic for an analog possessing the hematopoietic capabilities of natural G-CSF (if no receptor binding, signal transduction does not result from the presence of the analog). It is contemplated that one class of G-CSF analogs will possess the three dimensional core structure of a natural or recombinant (non-altered) G-CSF molecule, yet possess different characteristics, such as an increased ability to selectively stimulate neutrophils. Another class of G-CSF analogs are those with a different overall structure which diminishes the ability of a G-CSF analog molecule to bind to a G-CSF receptor, and possesses a diminished ability to selectively stimulate neutrophils as compared to non-altered natural or recombinant G-CSF.

For example, it is now known which moieties within the internal regions of the G-CSF molecule are hydrophobic, and, correspondingly, which moieties on the external portion of the G-CSF molecule are hydrophilic. Without knowledge of the overall three dimensional structure, preferably to the atomic level as provided herein, one could not forecast which alterations within this hydrophobic internal area would result in a change in the overall structural conformation of the molecule. An overall structural change could result in a functional change, such as lack of receptor binding, for example, and therefore, diminishment of biological activity as found in non-altered G-CSF. Another class of G-CSF analogs is therefore G-CSF analogs which possess the same hydrophobicity as (non-altered) natural or recombinant G-CSF. More particularly, another class of G-CSF analogs possesses the same hydrophobic moieties within the four helical bundle of its internal core as those hydrophobic moieties possessed by (non-altered) natural or recombinant G-CSF yet have a composition different from said non-altered natural or recombinant G-CSF.

Another example relates to external loops which are structures which connect the internal core (helices) of the G-CSF molecule. From the three dimensional structure -- including information regarding the spatial location of the amino acid residues -- one may forecast that certain changes in certain loops will not result in overall conformational changes. Therefore, another class of G-CSF analogs provided herein is that having an altered external loop but possessing the same overall structure as (non-altered) natural or recombinant G-CSF. More particularly, another class of G-CSF analogs provided herein are those having an altered external loop, said loop being selected from the loop present between helices A and B; between helices B and C; between helices C and D; between helices D and A, as those loops and helices are identified herein. More particularly, said loops, preferably the AB loop and/or the CD loop are altered to increase the half life of the molecule by stabilizing said loops. Such stabilization may be by connecting all or a portion of said loop(s) to a portion of an alpha helical bundle found in the core of a G-CSF (or analog) molecule. Such connection may be via beta sheet, salt bridge, disulfide bonds, hydrophobic interaction or other connecting means available to those skilled in the art, wherein such connecting means serves to stabilize said external loop or loops. For example, one may stabilize the AB or CD loops by connecting the AB loop to one of the helices within the internal region of the molecule.

The N-terminus also may be altered without change in the overall structure of a G-CSF molecule, because the N-terminus does not effect structural stability of the internal helices, and, although the external loops are preferred for modification, the same general statements apply to the N-terminus.

Additionally, such external loops may be the site(s) for chemical modification because in (non-altered) natural or recombinant G-CSF such loops are relatively flexible and tend not to interfere with receptor binding. Thus, there would be additional room for a chemical moiety to be directly attached (or indirectly

attached via another chemical moiety which serves as a chemical connecting means). The chemical moiety may be selected from a variety of moieties available for modification of one or more function of a G-CSF molecule. For example, an external loop may provide sites for the addition of one or more polymer which serves to increase serum half-life, such as a polyethylene glycol molecule. Such polyethylene glycol molecule(s) may be added wherein said loop is altered to include additional lysines which have reactive side groups to which polyethylene glycol moieties are capable of attaching. Other classes of chemical moieties may also be attached to one or more external loops, including but not limited to other biologically active molecules, such as receptors, other therapeutic proteins (such as other hematopoietic factors which would engender a hybrid molecule), or cytotoxic agents (such as diphtheria toxin). This list is of course not complete; one skilled in the art possessed of the desired chemical moiety will have the means to effect attachment of said desired moiety to the desired external loop. Therefore, another class of the present G-CSF analogs includes those with at least one alteration in an external loop wherein said alteration provides for the addition of a chemical moiety such as at least one polyethylene glycol molecule.

Deletions, such as deletions of sites recognized by proteins for degradation of the molecule, may also be effectual in the external loops. This provides alternative means for increasing half-life of a molecule otherwise having the G-CSF receptor binding and signal transduction capabilities (i.e., the ability to selectively stimulate the maturation of neutrophils). Therefore, another class of the present G-CSF analogs includes those with at least one alteration in an external loop wherein said alteration decreases the turnover of said analog by proteases. Preferred loops for such alterations are the AB loop and the CD loop. One may prepare an abbreviated G-CSF molecule by deleting a portion of the amino acid residues found in the external loops (identified in more detail below), said abbreviated G-CSF molecule may have additional advantages in preparation or in biological function.

Another example relates to the relative charges between amino acid residues which are in proximity to each other. As noted above, the G-CSF molecule contains a relatively tightly packed four helical bundle. Some of the faces on the helices face other helices. At the point (such as a residue) where a helix faces another helix, the two amino acid moieties which face each other may have the same charge, and thus tend to repel each other, which lends instability to the overall molecule. This may be eliminated by changing the charge (to an opposite charge or a neutral charge) of one or both of the amino acid moieties so that there is no repelling. Therefore, another class of G-CSF analogs includes those G-CSF analogs having been altered to modify instability due to surface interactions, such as electron charge location.

In another aspect, the present invention relates to methods for designing G-CSF analogs and related compositions and the products of those methods. The end products of the methods may be the G-CSF analogs as defined above or related compositions. For instance, the examples disclosed herein demonstrate (a) the effects of changes in the constituents (i.e., chemical moieties) of the G-CSF molecule on the G-CSF structure and (b) the effects of changes in structure on biological function. Essentially, therefore, another aspect of the present invention is a method for preparing a G-CSF analog comprising the steps of:

(a) viewing information conveying the three dimensional structure of a G-CSF molecule wherein the chemical moieties, such as each amino acid residue or each atom of each amino acid residue, of the G-CSF molecule are correlated with said structure;

(b) selecting from said information a site on a G-CSF molecule for alteration;

(c) preparing a G-CSF analog molecule having such alteration; and

(d) optionally, testing such G-CSF analog molecule for a desired characteristic.

One may use the here provided computer programs for a computer-based method for preparing a G-CSF analog. Another aspect of the present invention is therefore a computer based method for preparing a G-CSF analog comprising the steps of:

(a) providing computer expression of the three dimensional structure of a G-CSF molecule wherein the chemical moieties, such as each amino acid residue or each atom of each amino acid residue, of the G-CSF molecule are correlated with said structure;

(b) selecting from said computer expression a site on a G-CSF molecule for alteration;

(c) preparing a G-CSF molecule having such alteration; and

(d) optionally, testing such G-CSF molecule for a desired characteristic.

More specifically, the present invention provides a method for preparing a G-CSF analog comprising the steps of:

(a) viewing the three dimensional structure of a G-CSF molecule via a computer, said computer programmed (i) to express the coordinates of a G-CSF molecule in three dimensional space, and (ii) to allow for entry of information for alteration of said G-CSF expression and viewing thereof;

(b) selecting a site on said visual image of said G-CSF molecule for alteration;

(c) entering information for said alteration on said computer;

(d) viewing a three dimensional structure of said altered G-CSF molecule via said computer;
(e) optionally repeating steps (a)-(e);
(f) preparing a G-CSF analog with said alteration; and
(g) optionally testing said G-CSF analog for a desired characteristic.

In another aspect, the present invention relates to methods of using the present G-CSF analogs and related compositions and methods for the treatment or protection of mammals, either alone or in combination with other hematopoietic factors or drugs in the treatment of hematopoietic disorders. It is contemplated that one aspect of designing G-CSF analogs will be the goal of enhancing or modifying the characteristics non-modified G-CSF is known to have.

For example, the present analogs may possess enhanced or modified activities, so, where G-CSF is useful in the treatment of (for example) neutropenia, the present compositions and methods may also be of such use.

Another example is the modification of G-CSF for the purpose of interacting more effectively when used in combination with other factors particularly in the treatment of hematopoietic disorders. One example of such combination use is to use an early-acting hematopoietic factor (i.e., a factor which acts earlier in the hematopoiesis cascade on relatively undifferentiated cells) and either simultaneously or in seriatim use of a later-acting hematopoietic factor, such as G-CSF or analog thereof (as G-CSF acts on the CFU-GM lineage in the selective stimulation of neutrophils). The present methods and compositions may be useful in therapy involving such combinations or "cocktails" of hematopoietic factors.

The present compositions and methods may also be useful in the treatment of leukopenia, mylogenous leukemia, severe chronic neutropenia, aplastic anemia, glycogen storage disease, mucosistitis, and other bone marrow failure states. The present compositions and methods may also be useful in the treatment of hematopoietic deficits arising from chemotherapy or from radiation therapy. The success of bone marrow transplantation, or the use of peripheral blood progenitor cells for transplantation, for example, may be enhanced by application of the present compositions (proteins or nucleic acids for gene therapy) and methods. The present compositions and methods may also be useful in the treatment of infectious diseases, such in the context of wound healing, burn treatment, bacteremia, septicemia, fungal infections, endocarditis, osteopyelitis, infection related to abdominal trauma, infections not responding to antibiotics, pneumonia and the treatment of bacterial inflammation may also benefit from the application of the present compositions and methods. In addition, the present compositions and methods may be useful in the treatment of leukemia based upon a reported ability to differentiate leukemic cells. Welte et al., PNAS-USA 82: 1526-1530 (1985). Other applications include the treatment of individuals with tumors, using the present compositions and methods, optionally in the presence of receptors (such as antibodies) which bind to the tumor cells. For review articles on therapeutic applications, see Lieshhke and Burgess, N.Engl.J.Med. 327: 28-34 and 99-106 (1992) both of which are herein incorporated by reference.

The present compositions and methods may also be useful to act as intermediaries in the production of other moieties; for example, G-CSF has been reported to influence the production of other hematopoietic factors and this function (if ascertained) may be enhanced or modified via the present compositions and/or methods.

The compositions related to the present G-CSF analogs, such as receptors, may be useful to act as an antagonist which prevents the activity of G-CSF or an analog. One may obtain a composition with some or all of the activity of non-altered G-CSF or a G-CSF analog, and add one or more chemical moieties to alter one or more properties of such G-CSF or analog. With knowledge of the three dimensional conformation, one may forecast the best geographic location for such chemical modification to achieve the desired effect.

General objectives in chemical modification may include improved half-life (such as reduced renal, immunological or cellular clearance), altered bioactivity (such as altered enzymatic properties, dissociated bioactivities or activity in organic solvents), reduced toxicity (such as concealing toxic epitopes, compartmentalization, and selective biodistribution), altered immunoreactivity (reduced immunogenicity, reduced antigenicity or adjuvant action), or altered physical properties (such as increased solubility, improved thermal stability, improved mechanical stability, or conformational stabilization). See Francis, *Focus on Growth Factors* 3: 4-10 (May 1992) (published by Mediscript, Mountview Court, Friern Barnet Lane, London N20 OLD, UK).

The examples below are illustrative of the present invention and are not intended as a limitation. It is understood that variations and modifications will occur to those skilled in the art, and it is intended that the appended claims cover all such equivalent variations which come within the scope of the invention as claimed.

Detailed Description of the Drawings

FIGURE 1 is an illustration of the amino acid sequence of the 174 amino acid species of G-CSF with an additional N-terminal methionine (Seq. ID No.: 1) (Seq. ID No.: 2).

FIGURE 2 is an topology diagram of the crystalline structure of G-CSF, as well as hGH, pGH, GM-CSF, INF-B, IL-2, and IL-4. These illustrations are based on inspection of cited references. The length of secondary structural elements are drawn in proportion to the number of residues. A, B, C, and D helices are labeled according to the scheme used herein for G-CSF. For INF-β, the original labeling of helices is indicated in parentheses.

FIGURE 3 is an "ribbon diagram" of the three dimensional structure of G-CSF. Helix A is amino acid residues 11-39 (numbered according to Figure 1, above), helix B is amino acid residues 72-91, helix C is amino acid residues 100-123, and helix D is amino acid residues 143-173. The relatively short 3^{10} helix is at amino acid residues 45-48, and the alpha helix is at amino acid residues 48-53. Residues 93-95 form almost one turn of a left handed helix.

FIGURE 4 is a "barrel diagram" of the three dimensional structure of G-CSF. Shown in various shades of gray are the overall cylinders and their orientations for the three dimensional structure of G-CSF. The numbers indicate amino acid residue position according to FIGURE 1 above.

FIGURE 5 is a list of the coordinates used to generate a computer-aided visual image of the three-dimensional structure of G-CSF. The coordinates are set forth below. The columns correspond to separate field:

(i) Field 1 (from the left hand side) is the atom,

(ii) Field 2 is the assigned atom number,

(iii) Field 3 is the atom name (according to the periodic table standard nomenclature, with CB being carbon atom Beta, CG is Carbon atom Gamma, etc.);

(iv) Field 4 is the residue type (according to three letter nomenclature for amino acids as found in, e.g., Stryer, Biochemistry, 3d Ed., W.H. Freeman and Company, N.Y. 1988, inside back cover);

(v) Fields 5-7 are the x-axis, y-axis and z-axis positions of the atom;

(vi) Field 8 (often a "1.00") designates occupancy at that position;

(vii) Field 9 designates the B-factor;

(viii) Field 10 designates the molecule designation. Three molecules (designated a, b, and c) of G-CSF crystallized together as a unit. The designation a, b, or c indicates which coordinates are from which molecule. The number after the letter (1, 2, or 3) indicates the assigned amino acid residue position, with molecule A having assigned positions 10-175, molecule B having assigned positions 210-375, and molecule C having assigned positions 410-575. These positions were so designated so that there would be no overlap among the three molecules which crystallized together. (The "W" designation indicates water).

FIGURE 6 is a schematic representation of the strategy involved in refining the crystallization matrix for parameters involved in crystallization. The crystallization matrix corresponds to the final concentration of the components (salts, buffers and precipitants) of the crystallization solutions in the wells of a 24 well tissue culture plate. These concentrations are produced by pipetting the appropriate volume of stock solutions into the wells of the microtiter plate. To design the matrix, the crystallographer decides on an upper and lower concentration of the component. These upper and lower concentrations can be pipetted along either the rows (e.g., A1-A6, B1-B6, C1-C6 or D1-D6) or along the entire tray (A1-D6). The former method is useful for checking reproducibility of crystal growth of a single component along a limited number of wells, whereas the later method is more useful in initial screening. The results of several stages of refinement of the crystallization matrix are illustrated by a representation of three plates. The increase in shading in the wells indicates a positive crystallization result which, in the final stages, would be X-ray quality crystals but in the initial stages could be oil droplets, granular precipitates or small crystals approximately less than 0.05 mm in size. Part A represents an initial screen of one parameter in which the range of concentration between the first well (A1) and last well (D6) is large and the concentration increase between wells is calculated as (-(concentration A1)-(concentration D6))/23). Part B represents that in later stages of the crystallization matrix refinement of the concentration spread between A1 and D6 would be reduced which would result in more crystals formed per plate. Part C indicates a final stage of matrix refinement in which quality crystals are found in most wells of the plate.

Detailed Description of the Invention

The present invention grows out of the discovery of the three dimensional structure of G-CSF. This three dimensional structure has been expressed via computer program for stereoscopic viewing. By viewing this stereoscopically, structure-function relationships identified and G-CSF analogs have been designed and made.

The Overall Three Dimensional Structure of G-CSF

The G-CSF used to ascertain the structure was a non-glycosylated 174 amino acid species having an extra N-terminal methionine residue incident to bacterial expression. The DNA and amino acid sequence of this G-CSF are illustrated in FIGURE 1.

Overall, the three dimensional structure of G-CSF is predominantly helical, with 103 of the 175 residues forming a 4-alpha-helical bundle. The only other secondary structure is found in the loop between the first two long helices where a 4 residue 3^{10} helix is immediately followed by a 6 residue alpha helix. As shown in FIGURE 2, the overall structure has been compared with the structure reported for other proteins: growth hormone (Abdel-Meguid et al., PNAS-USA 84: 6434 (1987) and Vos et al., Science 255: 305-312 (1992)), granulocyte macrophage colony stimulating factor (Diederichs et al., Science 254: 1779-1782 (1991), interferon-β (Senda et al., EMBO J. 11: 3193-3201 (1992)), interleukin-2 (McKay Science 257: 1673-1677 (1992)) and interleukin-4 (Powers et al., Science 256: 1673-1677 (1992), and Smith et al., J. Mol. Biol. 224: 899-904 (1992)). Structural similarity among these growth factors occurs despite the absence of similarity in their amino acid sequences.

Presently, the structural information was correlation of G-CSF biochemistry, and this can be summarized as follows (with sequence position 1 being at the N-terminus):

Sequence Position	Description of Structure	Analysis
1-10	Extended chain	Deletion causes no loss of biological activity
Cys 18	Partially buried	Reactive with DTNB and Thimersosol but not with Iodo-acetate
34	Alternative splice site	Insertion reduces biological activity
20-47 (inclusive)	Helix A, first disulfide and portion of AB helix	Predicted receptor binding region based on neutralizing antibody data
20, 23, 24	Helix A	Single alanine mutation of residue(s) reduces biological activity. Predicted receptor binding (Site B).
165-175 (inclusive)	Carboxy terminus	Deletion reduces biological activity

This biochemical information, having been gleaned from antibody binding studies, see Layton et al., Biochemistry 266: 23815-23823 (1991), was superimposed on the three-dimensional structure in order to design G-CSF analogs. The design, preparation, and testing of these G-CSF analogs is described in Example 1 below.

EXAMPLE 1

This Example describes the preparation of crystalline G-CSF, the visualization of the three dimensional structure of recombinant human G-CSF via computer-generated image, the preparation of analogs, using site-directed mutagenesis or nucleic acid amplification methods, the biological assays and HPLC analysis used to analyze the G-CSF analogs, and the resulting determination of overall structure/function relationships. All cited publications are herein incorporated by reference.

A. Use of Automated Crystallization

The need for a three-dimensional structure of recombinant human granulocyte colony stimulating factor (r-hu-G-CSF), and the availability of large quantities of the purified protein, led to methods of crystal growth by incomplete factorial sampling and seeding. Starting with the implementation of incomplete factorial

crystallization described by Jancarik and Kim· J. Appl. Crystallogr. 24: 409 (1991) solution conditions that yielded oil droplets and birefringence aggregates were ascertained. Also, software and hardware of an automated pipetting system were modified to produce some 400 different crystallization conditions per day. Weber, J. Appl. Crystallogr. 20: 366-373 (1987). This procedure led to a crystallization solution which produced r-hu-G-CSF crystals.

The size, reproducibility and quality of the crystals was improved by a seeding method in which the number of "nucleation initiating units" was estimated by serial dilution of a seeding solution. These methods yielded reproducible growth of 2.0 mm r-hu-G-CSF crystals. The space group of these crystals is $P2_12_12_1$ with cell dimensions of a = 90 Å, b = 110 Å and c = 49 Å, and they diffract to a resolution of 2.0 Å.

1. Overall Methodology

To search for the crystallizing conditions of a new protein, Carter and Carter, J. Biol. Chem. 254: 122219-12223 (1979) proposed the incomplete factorial method. They suggested that a sampling of a large number of randomly selected, but generally probable, crystallizing conditions may lead to a successful combination of reagents that produce protein crystallization. This idea was implemented by Jancarik and Kim, J. Appl. Crystallogr. 24: 409(1991), who described 32 solutions for the initial crystallization trials which cover a range of pH, salts and precipitants. Here we describe an extension of their implementation to an expanded set of 70 solutions. To minimize the human effort and error of solution preparation, the method has been programmed for an automatic pipetting machine.

Following Weber's method of successive automated grid searching (SAGS), J.Cryst. Growth 90: 318-324(1988), the robotic system was used to generate a series of solutions which continually refined the crystallization conditions of temperature, pH, salts and precipitant. Once a solution that could reproducibly grow crystals was determined, a seeding technique which greatly improved the quality of the crystals was developed. When these methods were combined, hundreds of diffraction quality crystals (crystals diffracting to at least about 2.5 Angstroms, preferably having at least portions diffracting to below 2 Angstroms, and more preferably, approximately 1 Angstrom) were produced in a few days.

Generally, the method for crystallization, which may be used with any protein one desires to crystallize, comprises the steps of:

(a) combining aqueous aliquots of the desired protein with either (i) aliquots of a salt solution, each aliquot having a different concentration of salt; or (ii) aliquots of a precipitant solution, each aliquot having a different concentration of precipitant, optionally wherein each combined aliquot is combined in the presence of a range of pH;

(b) observing said combined aliquots for precrystalline formations, and selecting said salt or precipitant combination and said pH which is efficacious in producing precrystalline forms, or, if no precrystalline forms are so produced, increasing the protein starting concentration of said aqueous aliquots of protein;

(c) after said salt or said precipitant concentration is selected, repeating step (a) with said previously unselected solution in the presence of said selected concentration; and

(d) repeating step (b) and step (a) until a crystal of desired quality is obtained.

The above method may optionally be automated, which provides vast savings in time and labor. Preferred protein starting concentrations are between 10mg/ml and 20mg/ml, however this starting concentration will vary with the protein (the G-CSF below was analyzed using 33mg/ml). A preferred range of salt solution to begin analysis with is (NaCl) of 0-2.5M. A preferred precipitant is polyethylene glycol 8000, however, other precipitants include organic solvents (such as ethanol), polyethylene glycol molecules having a molecular weight in the range of 500-20,000, and other precipitants known to those skilled in the art. The preferred pH range is pH 4.5 , 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, and 9.0. Precrystallization forms include oils, birefringement precipitants, small crystals (< approximately 0.05 mm), medium crystals (approximately 0.5 to .5 mm) and large crystals (> approximately 0.5 mm). The preferred time for waiting to see a crystalline structure is 48 hours, although weekly observation is also preferred, and generally, after about one month, a different protein concentration is utilized (generally the protein concentration is increased). Automation is preferred, using the Accuflex system as modified. The preferred automation parameters are described below.

Generally, protein with a concentration between 10 mg/ml and 20 mg/ml was combined with a range of NaCl solutions from 0-2.5 M, and each such combination was performed (separately) in the presence of the above range of concentrations. Once a precrystallization structure is observed, that salt concentration and pH range are optimized in a separate experiment, until the desired crystal quality is achieved. Next, the precipitant concentration, in the presence of varying levels of pH is also optimized. When both are optimized, the optimal conditions are performed at once to achieve the desired result (this is diagrammed in

FIGURE 6).

a. Implementation of an automated pipetting system

Drops and reservoir solutions were prepared by an Accuflex pipetting system (ICN Pharmaceuticals, Costa Mesa, CA) which is controlled by a personal computer that sends ASCII codes through a standard serial interface. The pipetter samples six different solutions by means of a rotating valve and pipettes these solutions onto a plate whose translation in a x-y coordinate system can be controlled. The vertical component of the system manipulates a syringe that is capable both of dispensing and retrieving liquid.

The software provided with the Accuflex was based on the SAGS method as proposed by Cox and Weber, J.Appl. Crystallogr. 20: 366-373 (1987). This method involves the systematic variation of two major crystallization parameters, pH and precipitant concentration, with provision to vary two others. While building on these concepts, the software used here provided greater flexibility in the design and implementation of the crystallization solutions used in the automated grid searching strategy. As a result of this flexibility the present software also created a larger number of different solutions. This is essential for the implementation of the incomplete factorial method as described in that section below.

To improve the speed and design of the automated grid searching strategy, the Accuflex pipetting system required software and hardware modifications. The hardware changes allowed the use of two different micro-liter trays, one used for handling drop and one used for sitting drop experiments, and a Plexiglas tray which held 24 additional buffer, salt and precipitant solutions. These additional solutions expanded the grid of crystallizing conditions that could be surveyed.

To utilize the hardware modifications, the pipetting software was written in two subroutines; one subroutine allows the crystallographer to design a matrix of crystallization solutions based on the concentrations of their components and the second subroutine to translate these concentrations into the computer code which pipettes the proper volumes of the solutions into the crystallization trays. The concentration matrices can be generated by either of two programs. The first program (MRF, available from Amgen, Inc., Thousand Oaks, CA) refers to a list of stock solution concentrations supplied by the crystallographer and calculates the required volume to be pipette to achieve the designated concentration. The second method, which is preferred, incorporates a spread sheet program (Lotus) which can be used to make more sophisticated gradients of precipitants or pH. The concentration matrix created by either program is interpreted by the control program (SUX, a modification of the program found in the Accuflex pipetter originally and available from Amgen, Inc., Thousand Oaks, CA) and the wells are filled accordingly.

b. Implementation of the Incomplete Factorial Method

The convenience of the modified pipetting system for preparing diverse solutions improved the implementation of an expanded incomplete factorial method. The development of a new set of crystallization solutions having "random" components was generated using the program INFAC, Carter et al., J.Cryst. Growth 90: 60-73(1988) which produced a list containing 96 random combinations of one factor from three variables. Combinations of calcium and phosphate which immediately precipitated were eliminated, leaving 70 distinct combinations of precipitants, salts and buffers. These combinations were prepared using the automated pipetter and incubated for 1 week. The mixtures were inspected and solutions which formed precipitants were prepared again with lower concentrations of their components. This was repeated until all wells were clear of precipitant.

c. Crystallization of r-hu-G-CSF

Several different crystallization strategies were used to find a solution which produced x-ray quality crystals. These strategies included the use of the incomplete factorial method, refinement of the crystallization conditions using successive automated grid searches (SAGS), implementation of a seeding technique and development of a crystal production procedure which yielded hundreds of quality crystals overnight. Unless otherwise noted the screening and production of r-hu-G-CSF crystals utilized the hanging drop vapor diffusion method. Atinsen et al., Physical principles of protein crystallization. In: Eisenberg (ed.), Advances in Protein Chemistry 41: 1-33 (1991).

The initial screening for crystallization conditions of r-hu-G-CSF used the Jancarik and Kim, J.Appl.Crystallogr. 24: 409(1991) incomplete factorial method which resulted in several solutions that produced "precrystallization" results. These results included birefringent precipitants, oils and very small crystals (< .05 mm). These precrystallizations solutions then served as the starting points for systematic

screening.

The screening process required the development of crystallization matrices. These matrices corresponded to the concentration of the components in the crystallization solutions and were created using the IBM-PC based spread sheet Lotus™ and implemented with the modified Accuflex pipetting system. The strategy in designing the matrices was to vary one crystallization condition (such as salt concentration) while holding the other conditions such as pH, and precipitant concentration constant. At the start of screening, the concentration range of the varied condition was large but the concentration was successively refined until all wells in the micro-titer tray produced the same crystallization result. These results were scored as follows: crystals, birefringement precipitate, granular precipitate, oil droplets and amorphous mass. If the concentration of a crystallization parameter did not produce at least a precipitant, the concentration of that parameter was increased until a precipitant formed. After each tray was produced, it was left undisturbed for at least two days and then inspected for crystal growth. After this initial screening, the trays were then inspected on a weekly basis.

From this screening process, two independent solutions with the same pH and precipitant but differing in salts ($MgCl$, $LiSO_4$) were identified which produced small (0.1 x 0.05 x 0.05 mm) crystals. Based on these results, a new series of concentration matrices were produced which varied $MgCl$ with respect to $LiSO_4$ while keeping the other crystallization parameters constant. This series of experiments resulted in identification of a solution which produced diffraction quality crystals (> approximately 0.5 mm) in about three weeks. To find this crystallization growth solution (100 mM Mes pH 5.8, 380 mM $MgCl_2$, 220 mM $LiSO4$ and 8% PEG 8k) approximately 8,000 conditions had been screened which consumed about 300 mg of protein.

The size of the crystals depended on the number of crystals forming per drop. Typically 3 to 5 crystals would be formed with average size of (1.0 x 0.7 x 0.7 mm). Two morphologies which had an identical space group ($P2_12_12_1$) and unit cell dimensions a = 90.2, b = 110.2, c = 49.5 were obtained depending on whether or not seeding (see below) was implemented. Without seeding, the r-hu-G-CSF crystals had one long flat surface and rounded edges.

When seeding was employed, crystals with sharp faces were observed in the drop within 4 to 6 hours (0.05 by 0.05 by 0.05 mm). Within 24 hours, crystals had grown to (0.7 by 0.7 by 0.7 mm) and continued to grow beyond 2 mm depending on the number of crystals forming in the drop.

d. Seeding and determination of nucleation initiation sites.

The presently provided method for seeding crystals establishes the number of nucleation initiation units in each individual well used (here, after the optimum conditions for growing crystals had been determined). The method here is advantageous in that the number of "seeds" affects the quality of the crystals, and this in turn affects the degree of resolution. The present seeding here also provides advantages in that with seeding, G-CSF crystal grows in a period of about 3 days, whereas without seeding, the growth takes approximately three weeks.

In one series of production growth (see methods), showers of small but well defined crystals were produced overnight (<0.01 x 0.01 x0.01 mm). Crystallization conditions were followed as described above except that a pipette tip employed in previously had been reused. Presumably, the crystal showering effect was caused by small nucleation units which had formed in the used tip and which provided sites of nucleation for the crystals. Addition of a small amount (0.5 ul) of the drops containing the crystal showers to a new drop under standard production growth conditions resulted in a shower of crystals overnight. This method was used to produce several trays of drops containing crystal showers which we termed "seed stock".

The number of nucleation initiation units (NIU) contained within the "seed stock" drops was estimated to attempt to improve the reproducibility and quality of the r-hu-GCSF crystals. To determine the number of NIU in the "seed stock", an aliquot of the drop was serially diluted along a 96 well microtiter plate. The microtiter plate was prepared by adding 50 ul of a solution containing equal volumes of r-hu-G-CSF (33 mg/ml) and the crystal growth solution (described above) in each well. An aliquot (3 ul) of one of the "seed stock" drops was transferred to the first well of the microtiter plate. The solution in the well was mixed and 3 ul was then transferred to the next well along the row of the microtiter plate. Each row of the microtiter plate was similarly prepared and the tray was sealed with plastic tape. Overnight, small crystals formed in the bottom of the wells of the microtiter plate and the number of crystals in the wells were correlated to the dilution of the original "seed stock". To produce large single crystals, the "seed stock" drop was appropriately diluted into fresh CGS and then an aliquot of this solution containing the NIU was transferred to a drop

Once crystallization conditions had been optimized, crystals were grown in a production method in which 3 ml each of CGS and r-hu-G-CSF (33 mg/ml) were mixed to create 5 trays (each having 24 wells). This method included the production of the refined crystallization solution in liter quantities, mixing this solution with protein and placing the protein/crystallization solution in either hanging drop or sitting drop trays. This process typically yielded 100 to 300 quality crystals (>0.5 mm) in about 5 days.

e. Experimental Methods

Materials

Crystallographic information was obtained starting with r-hu-met-G-CSF with the amino acid sequence as provided in FIGURE 1 with a specific activity of 1.0 +/- 0.6 x 10^8 U/mg (as measured by cell mitogenesis assay in a 10 mM acetate buffer at pH 4.0 (in Water for Injection) at a concentration of approximately 3 mg/ml solution was concentrated with an Amicon concentrator at 75 psi using a YM10 filter. The solution was typically concentrated 10 fold at 4°C and stored for several months.

Initial Screening

Crystals suitable for X-ray analysis were obtained by vapor-diffusion equilibrium using hanging drops. For preliminary screening, 7 ul of the protein solution at 33 mg/ml (as prepared above) was mixed with an equal volume of the well solution, placed on siliconized glass plates and suspended over the well solution utilizing Linbro tissue culture plates (Flow Laboratories, McLean, Va). All of the pipetting was performed with the Accuflex pipetter, however, trays were removed from the automated pipetter after the well solutions had been created and thoroughly mixed for at least 10 minutes with a table top shaker. The Linbro trays were then returned to the pipetter which added the well and protein solutions to the siliconized cover slips. The cover slips were then inverted and sealed over 1 ml of the well solutions with silicon grease.

The components of the automated crystallization system are as follows. A PC-DOS computer system was used to design a matrix of crystallization solutions based on the concentration of their components. These matrices were produced with either MRF of the Lotus spread sheet (described above). The final product of these programs is a data file. This file contains the information required by the SUX program to pipette the appropriate volume of the stock solutions to obtain the concentrations described in the matrices. The SUX program information was passed through a serial I/O port and used to dictate to the Accuflex pipetting system the position of the valve relative to the stock solutions, the amount of solution to be retrieved, and then pipetted into the wells of the microtiter plates and the X-Y position of each well (the column/row of each well). Addition information was transmitted to the pipetter which included the Z position (height) of the syringe during filling as well as the position of a drain where the system pauses to purge the syringe between fillings of different solutions. The 24 well microtiter plate (either Linbro or Cryschem) and cover slip holder was placed on a plate which was moved in the X-Y plane. Movement of the plate allowed the pipetter to position the syringe to pipette into the wells. It also positioned the coverslips and vials and extract solutions from these sources. Prior the pipetting, the Linbro microtiter plates had a thin film of grease applied around the edges of the wells. After the crystallization solutions were prepared in the wells and before they were transferred to the cover slips, the microtiter plate was removed from the pipetting system, and solutions were allowed to mix on a table top shaker for ten minutes. After mixing, the well solution was either transferred to the cover slips (in the case of the hanging drop protocol) or transferred to the middle post in the well (in the case of the sitting drop protocol). Protein was extracted from a vial and added to the coverslip drop containing the well solution (or to the post). Plastic tape was applied to the top of the Cryschem plate to seal the wells.

Production Growth

Once conditions for crystallization had been optimized, crystal growth was performed utilizing a "production" method. The crystallization solution which contained 100 mM Mes pH 5.8, 380 mM MgCl2, 220 mM LiSO4, and 8% PEG 8K was made in 1 liter quantities. Utilizing an Eppindorf syringe pipetter, 1 ml aliquots of this solution were pipetted into each of the wells of the Linbro plate. A solution containing 50% of this solution and 50% G-CSF (33 mg/ml) was mixed and pipetted onto the siliconized cover slips. Typical volumes of these drops were between 50 and 100 ul and because of the large size of these drops, great care was taken in flipping the coverslips and suspending the drops over the wells.

Data Collection

The structure has been refined with X-PLOR (Bruniger, X-PLOR version 3.0. A system for crystallography and NMR, Yale University, New Haven CT) against 2.2Å data collected on an R-AXIS (Molecular Structure, Corp. Houston, TX) imaging plate detector.

f. Observations

As an effective recombinant human therapeutic, r-hu-G-CSF has been produced in large quantities and gram levels have been made available for structural analysis. The crystallization methods provided herein are likely to find other applications as other proteins of interest become available. This method can be applied to any crystallographic project which has large quantities of protein (approximately >200 mg). As one skilled in the art will recognize, the present materials and methods may be modified and equivalent materials and methods may be available for crystallization of other proteins.

B. Computer Program For Visualizing The Three Dimensional Structure of G-CSF

Although diagrams, such as those in the Figures herein, are useful for visualizing the three dimensional structure of G-CSF, a computer program which allows for stereoscopic viewing of the molecule is contemplated as preferred. This stereoscopic viewing, or "virtual reality" as those in the art sometimes refer to it, allows one to visualize the structure in its three dimensional form from every angle in a wide range of resolution, from macromolecular structure down to the atomic level. The computer programs contemplated herein also allow one to change perspective of the viewing angle of the molecule, for example by rotating the molecule. The contemplated programs also respond to changes so that one may, for example, delete, add, or substitute one or more images of atoms, including entire amino acid residues, or add chemical moieties to existing or substituted groups, and visualize the change in structure.

Other computer based systems may be used; the elements being: (a) a means for entering information, such as orthogonal coordinates or other numerically assigned coordinates of the three dimensional structure of G-CSF; (b) a means for expressing such coordinates, such as visual means so that one may view the three dimensional structure and correlate such three dimensional structure with the composition of the G-CSF molecule, such as the amino acid composition; (c) optionally, means for entering information which alters the composition of the G-CSF molecule expressed, so that the image of such three dimensional structure displays the altered composition.

The coordinates for the preferred computer program used are presented in FIGURE 5. The preferred computer program is Insight II, version 4, available from Biosym in San Diego, CA. For the raw crystallographic structure, the observed intensities of the diffraction data ("F-obs") and the orthogonal coordinates are also deposited in the Protein Data Bank, Chemistry Department, Brookhaven National Laboratory, Upton, New York 119723, USA and these are herein incorporated by reference.

Once the coordinates are entered into the Insight II program, one can easily display the three dimensional G-CSF molecule representation on a computer screen. The preferred computer system for display is Silicon Graphics 320 VGX (San Diego, CA). For stereoscopic viewing, one may wear eyewear (Crystal Eyes, Silicon Graphics) which allows one to visualize the G-CSF molecule in three dimensions stereoscopically, so one may turn the molecule and envision molecular design.

Thus, the present invention provides a method of designing or preparing a G-CSF analog with the aid of a computer comprising:

(a) providing said computer with the means for displaying the three dimensional structure of a G-CSF molecule including displaying the composition of moieties of said G-CSF molecule, preferably displaying the three dimensional location of each amino acid, and more preferably displaying the three dimensional location of each atom of a G-CSF molecule;

(b) viewing said display;

(c) selecting a site on said display for alteration in the composition of said molecule or the location of a moiety; and

(d) preparing a G-CSF analog with such alteration.

The alteration may be selected based on the desired structural characteristics of the end-product G-CSF analog, and considerations for such design are described in more detail below. Such considerations include the location and compositions of hydrophobic amino acid residues, particularly residues internal to the helical structures of a G-CSF molecule which residues, when altered, alter the overall structure of the internal core of the molecule and may prevent receptor binding; the location and compositions of external

loop structures, alteration of which may not affect the overall structure of the G-CSF molecule.

FIGURES 2-4 illustrate the overall three dimensional conformation in different ways. The topological diagram, the ribbon diagram, and the barrel diagram all illustrate aspects of the conformation of G-CSF.

FIGURE 2 illustrates a comparison between G-CSF and other molecules. There is a similarity of architecture, although these growth factors differ in the local conformations of their loops and bundle geometrics. The up-up-down-down topology with two long crossover connections is conserved, however, among all six of these molecules, despite the dissimilarity in amino acid sequence.

FIGURE 3 illustrates in more detail the secondary structure of recombinant human G-CSF. This ribbon diagram illustrates the handedness of the helices and their positions relative to each other.

FIGURE 4 illustrates in a different way the conformation of recombinant human G-CSF. This "barrel" diagram illustrates the overall architecture of recombinant human G-CSF.

C. Preparation of Analogs Using M13 Mutagenesis

This example relates to the preparation of G-CSF analogs using site directed mutagenesis techniques involving the single stranded bacteriophage M13, according to methods published in PCT Application No. WO 85/00817 (Souza et al., published February 28, 1985, herein incorporated by reference). This method essentially involves using a single-stranded nucleic acid template of the non-mutagenized sequence, and binding to it a smaller oligonucleotide containing the desired change in the sequence. Hybridization conditions allow for non-identical sequences to hybridize and the remaining sequence is filled in to be identical to the original template. What results is a double stranded molecule, with one of the two strands containing the desired change. This mutagenized single strand is separated, and used itself as a template for its complementary strand. This creates a double stranded molecule with the desired change.

The original G-CSF nucleic acid sequence used is presented in FIGURE 1, and the oligonucleotides containing the mutagenized nucleic acid(s) are presented in Table 2. Abbreviations used herein for amino acid residues and nucleotides are conventional, see Stryer, Biochemistry, 3d Ed., W.H. Freeman and Company, N.Y., N.Y. 1988, inside back cover.

The original G-CSF nucleic acid sequence was first placed into vector M13mp21. The DNA from single stranded phage M13mp21 containing the original G-CSF sequence was then isolated, and resuspended in water. For each reaction, 200 ng of this DNA was mixed with a 1.5 pmole of phosphorylated oligonucleotide (Table 2) and suspended in 0.1M Tris, 0.01M $MgCl_2$, 0.005M DTT, 0.1mM ATP, pH 8.0. The DNAs were annealed by heating to 65°C and slowly cooling to room temperature.

Once cooled, 0.5mM of each ATP, dATP, dCTP, dGTP, TTP, 1 unit of T4 DNA ligase and 1 unit of Klenow fragment of E. coli polymerase 1 were added to the 1 unit of annealed DNA in 0.1M Tris, 0.025M NaCl, 0.01M $MgCl_2$, 0.01M DTT, pH 7.5.

The now double stranded, closed circular DNA was used to transfect E. coli without further purification. Plaques were screened by lifting the plaques with nitrocellulose filters, and then hybridizing the filters with single stranded DNA end-labeled with P^{32} for 1 hour at 55-60°C. After hybridization, the filters were washed at 0-3°C below the melt temperature of the oligo (2°C for A-T, 4°C for G-C) which selectively left autoradiography signals corresponding to plaques with phage containing the mutated sequence. Positive clones were confirmed by sequencing.

Set forth below are the oligonucleotides used for each G-CSF analog prepared via the M13 mutagenesis method. The nomenclature indicates the residue and the position of the original amino acid (e.g., Lysine at position 17), and the residue and position of the substituted amino acid (e.g., arginine 17). A substitution involving more than one residue is indicated via superscript notation, with commas between the noted positions or a semicolon indicating different residues. Deletions with no substitutions are so noted. The oligonucleotide sequences used for M13-based mutagenesis are next indicated; these oligonucleotides were manufactured synthetically, although the method of preparation is not critical, any nucleic acid synthesis method and/or equipment may be used. The length of the oligo is also indicated. As indicated above, these oligos were allowed to contact the single stranded phage vector, and then single nucleotides were added to complete the G-CSF analog nucleic acid sequence.

Table 2

G-CSF ANALOGS	SEQUENCES (5'-> 3')	Length (nucleotide)	Seq. ID
Lys^{17}->Arg^{17}	CTT TCT GCT GCG TTG TCT GGA ACA	24	3
Lys^{24}->Arg^{24}	ACA GGT TCG TCG TAT CCA GGG TG	23	4
Lys^{35}->Arg^{35}	CAC TGC AAG AAC GTC TGT GCG CT	23	5
Lys^{41}->Arg^{41}	CGC TAC TTA CCG TCT GTG CCA TC	23	6
$Lys^{17,24,35}$->	CTT TCT GCT GCG TTG TCT GGA ACA	24	7
$Arg^{17,24,35}$	ACA GGT TCG TCG TAT CCA GGG TG	23	8
	CAC TGC AAG AAC GTC TGT GCG CT	23	9
$Lys^{17,24,41}$->	CTT TCT GCT GCG TTG TCT GGA ACA	24	10
$Arg^{17,24,41}$	ACA GGT TCG TCG TAT CCA GGG TG	23	11
	CGC TAC TTA CCG TCT GTC CCA TC	23	12
$Lys^{17,35,41}$->	CTT TCT GCT GCG TTG TCT GGA ACA	24	13
$Arg^{17,35,41}$	CAC TGC AAG AAC GTC TGT GCG CT	23	14
	CGC TAC TTA CCG TCT GTG CCA TC	23	15
$Lys^{24,35,41}$->	ACA GGT TCG TCG TAT CCA GGG TG	23	16
$Arg^{24,35,41}$	CAC TGC AAG AAC GTC TGT GCG CT	23	17
	CGC TAC TTA CCG TCT GTG CCA TC	23	18

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Table 2 (con't)

G-CSF ANALOGS	SEQUENCES(5'-> 3')	Length(nucleotide)	Seq. ID
$Lys^{17,24,35,41}$->	CTT TCT GCT GCG TTG TCT GGA ACA	24	19
$Arg^{17,24,35,41}$	ACA GGT TCG TCG TAT CCA GGG TG	23	20
	CAC TGC AAG AAC GTC TGT GCG CT	23	21
	CGC TAC TTA CCG TCT GTG CCA TC	23	22
Cys^{18}->Ala^{18}	TCT GCT GAA AGC TCT GGA ACA GG	23	23
Gln^{68}->Glu^{68}	CTT GTC CAT CTG AAG CTC TTC AG	23	24
$Cys^{37,43}$->	GAA AAA CTG TCC GCT ACT TAC AAA	37	25
$Ser^{37,43}$	CTG TCC CAT CCG G		
Gln^{26}->Ala^{26}	TTC GTA AAA TCG CGG GTG ACG G	22	26
Gln^{174}->Ala^{174}	TCA TCT GGC TGC GCC GTA ATA G	22	27
Arg^{170}->Ala^{170}	CCG TGT TCT GGC TCA TCT GGC T	22	28
Arg^{167}->Ala^{167}	GAA GTA TCT TAC GCT GTT CTG CGT	24	29
Deletion 167	GAA GTA TCT TAC TAA GTT CTG CGT C	25	30
Lys^{41}->Ala^{41}	CGC TAC TTA CGC ACT GTG CCA T	22	31
His^{44}->Lys^{44}	CAA ACT GTG CAA GCC GGA AGA G	22	32
Glu^{47}->Ala^{47}	CAT CCG GAA GCA CTG GTA CTG C	22	33

Table 2 (con't)

G-CSF ANALOGS	SEQUENCES(5'-> 3')	Length(nucleotide)	Seq. ID
Arg^{23}->Ala^{23}	GGA ACA GGT TGC TAA AAT CCA GG	23	34
Lys^{24}->Ala^{24}	GAA CAG GTT CGT GCG ATC CAG GGT G	25	35
Glu^{20}->Ala^{20}	GAA ATG TCT GGC ACA GGT TCG T	22	36
Asp^{28}->Ala^{28}	TCC AGG GTG CCG GTG CTG C	19	37
Met^{127}->Glu^{127}	AAG AGC TCG GTG AGG CAC CAG CT	23	38
Met^{138}->Glu^{138}	CTC AAG GTG CTG AGC CGG CAT TC	23	39
Met^{127}->Leu^{127}	GAG CTC GGT CTG GCA CCA GC	20	40
Met^{138}->Leu^{138}	TCA AGG TGC TCT GCC GGC ATT	21	41
Ser^{13}->Ala^{13}	TCT GCC GCA AGC CTT TCT GCT GA	23	42
Lys^{17}->Ala^{17}	CTT TCT GCT GGC ATG TCT GGA ACA	24	43
Gln^{121}->Ala^{121}	CTA TTT GGC AAG CGA TGG AAG AGC	24	44
Glu^{124}->Ala^{124}	CAG ATG GAA GCG CTC GGT ATG	21	45

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Table 2 (con't)

G-CSF ANALOGS	SEQUENCES(5'-> 3')	Length(nucleotide)	Seq. ID
Met^{127},138-> Leu^{127},138	GAG CTC GGT CTG GCA CCA GC TCA AGG TGC TCT GCC GGC ATT	20 21	46 47
**Glu^{20}->Ala^{20}; Ser^{13}->Gly^{13}	GAA ATG TCT GGC ACA GGT TCG T	22	48

** This analog came about during the preparation of G-CSF analog Glu^{20}->Ala^{20}. As several clones were being sequenced to identify the Glu^{20}->Ala20 analog, the Glu^{20}->Ala^{20}; Ser^{13}->Gly13 analog was identified. This double mutant was the result of an in vitro Klenow DNA polymerase reaction mistake.

D. Preparation of G-CSF Analogs Using DNA Amplification

This example relates to methods for producing G-CSF analogs using a DNA amplification technique. Essentially, DNA encoding each analog was amplified in two separate pieces, combined, and then the total

sequence itself amplified. Depending upon where the desired change in the original G-CSF DNA was to be made, internal primers were used to incorporate the change, and generate the two separate amplified pieces. For example, for amplification of the 5' end of the desired analog DNA, a 5' flanking primer (complementary to a sequence of the plasmid upstream from the G-CSF original DNA) was used at one end of the region to be amplified, and an internal primer, capable of hybridizing to the original DNA but incorporating the desired change, was used for priming the other end. The resulting amplified region stretched from the 5' flanking primer through the internal primer. The same was done for the 3' terminus, using a 3' flanking primer (complementary to a sequence of the plasmid downstream from the G-CSF original DNA) and an internal primer complementary to the region of the intended mutation. Once the two "halves" (which may or may not be equal in size, depending on the location of the internal primer) were amplified, the two "halves" were allowed to connect. Once connected, the 5' flanking primer and the 3' flanking primer were used to amplify the entire sequence containing the desired change.

If more than one change is desired, the above process may be modified to incorporate the change into the internal primer, or the process may be repeated using a different internal primer. Alternatively, the gene amplification process may be used with other methods for creating changes in nucleic acid sequence, such as the phage based mutagenesis technique as described above. Examples of process for preparing analogs with more than one change are described below.

To create the G-CSF analogs described below, the template DNA used was the sequence as in FIGURE 1 plus certain flanking regions (from a plasmid containing the G-CSF coding region). These flanking regions were used as the 5' and 3' flanking primers and are set forth below. The amplification reactions were performed in 40 ul volumes containing 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1 mg/ml gelatin, pH 8.3 at 20°C. The 40 ul reactions also contained 0.1mM of each dNTP, 10 pmoles of each primer, and 1 ng of template DNA. Each amplification was repeated for 15 cycles. Each cycle consisted of 0.5 minutes at 94°C, 0.5 minutes at 50°C, and 0.75 minutes at 72°C. Flanking primers were 20 nucleotides in length and internal primers were 20 to 25 nucleotides in length. This resulted in multiple copies of double stranded DNA encoding either the front portion or the back portion of the desired G-CSF analog.

For combining the two "halves," one fortieth of each of the two reactions was combined in a third DNA amplification reaction. The two portions were allowed to anneal at the internal primer location, as their ends bearing the mutation were complementary, and following a cycle of polymerization, give rise to a full length DNA sequence. Once so annealed, the whole analog was amplified using the 5' and 3' flanking primers. This amplification process was repeated for 15 cycles as described above.

The completed, amplified analog DNA sequence was cleaved with XbaI and XhoI restriction endonuclease to produce cohesive ends for insertion into a vector. The cleaved DNA was placed into a plasmid vector, and that vector was used to transform *E. coli*. Transformants were challenged with kanamycin at 50 ug/ml and incubated at 30°C. Production of G-CSF analog protein was confirmed by polyacrylamide gel electrophoresis of a whole cell lysate. The presence of the desired mutation was confirmed by DNA sequence analysis of plasmid purified from the production isolate. Cultures were then grown, and cells were harvested, and the G-CSF analogs were purified as set forth below.

Set forth below in Table 3 are the specific primers used for eachanalog made using gene amplification.

Table 3

Analog Seq. ID	Internal Primer(5'->3')	
His^{44}->Ala^{44}	5'primer-TTCCGGAGCGCACAGTTTG	49
	3'primer-CAAACTGTGGGCTCCGGAAGAGC	50
Thr^{117}->Ala^{117}	5'primer-ATGCCAAATTGCAGTAGCAAAG	51
	3'primer-CTTTGCTACTGCAATTTGGCAACA	52
Asp^{110}->Ala^{110}	5'primer-ATCAGCTACTGCTAGCTGCAGA	53
	3'primer-TCTGCAGCTAGCAGTAGCTGACT	54
Gln^{21}->Ala^{21}	5'primer-TTACGAACCGCTTCCAGACATT	55
	3'primer-AATGTCTGGAAGCGGTTCGTAAAAT	56
Asp^{112}->Ala^{113}	5'primer-GTAGCAAATGCAGCTACATCTA	57
	3'primer-TAGATGTAGCTGCATTTGCTACTAC	58
His^{53}->Ala^{53}	5'primer-CCAAGAGAAGCACCCAGCAG	59
	3'primer-CTGCTGGGTGCTTCTCTTGGGA	60
For each analog, the following 5' flanking primer was used:		
5'-CACTGGCGGTGATAATGAGC		61
For each analog, the following 3' flanking primer was used:		
3'-GGTCATTACGGACCGGATC		62

1. Construction of Double Mutation

To make G-CSF analog $Gln^{12,21}$->$Glu^{12,21}$, two separate DNA amplifications were conducted to create the two DNA mutations. The template DNA used was the sequence as in FIGURE 1 plus certain flanking regions (from a plasmid containing the G-CSF coding region). The precise sequences are listed below. Each of the two DNA amplification reactions were carried out using a Perkin Elmer/Cetus DNA Thermal Cycler. The 40 ul reaction mix consisted of 1X PCR Buffer (Cetus), 0.2 mM each of the 4 dXTPs (Cetus), 50 pmoles of each primer oligonucleotide, 2 ng of G-CSF template DNA (on a plasmid vector), and 1 unit of Taq polymerase (Cetus). The amplification process was carried out for 30 cycles. Each cycle consisted of 1minute at 94 °C, 2 minutes at 50 °C, and 3 minutes at 72 °C.

DNA amplification "A" used the oligonucleotides:

5' CCACTGGCGGTGATACTGAGC 3' (Seq. ID 63) and

5' AGCAGAAAGCTTTCCGGCAGAGAAGAAGCAGGA 3' (Seq. ID 64)

DNA amplification "B" used the oligonucleotides: 5' GCCGCAAAGCTTTCTGCTGAAATGTCTG-GAAGAGGTTCGTAAAATCCAGGGTGA 3' (Seq. ID 65) and

5' CTGGAATGCAGAAGCAAATGCCGGCATAGCACCTTCAGTCGGTTGCAGAGCTGGTGCCA 3' (Seq. ID 66)

From the 109 base pair double stranded DNA product obtained after DNA amplification "A", a 64 base pair XbaI to HindIII DNA fragment was cut and isolated that contained the DNA mutation Gln^{12}->Glu^{12}. From the 509 base pair double stranded DNA product obtained after DNA amplification "B", a 197 base pair HindIII to BsmI DNA fragment was cut and isolated that contained the DNA mutation Gln^{21}->Glu^{21}.

The "A" and "B" fragments were ligated together with a 4.8 kilo-base pair XbaI to BsmI DNA plasmid vector fragment. The ligation mix consisted of equal molar DNA restriction fragments, ligation buffer (25 mM Tris-HCl pH 7.8, 10 mM $MgCl_2$, 2 mM DTT, 0.5 mM rATP, and 100 ug/ml BSA) and T4 DNA ligase and was incubated overnight at 14 °C. The ligated DNA was then transformed into E. coli FM5 cells by electroporation using a Bio Rad Gene Pulsar apparatus (BioRad, Richmond, CA). A clone was isolated and the plasmid construct verified to contain the two mutations by DNA sequencing. This 'intermediate' vector also contained a deletion of a 193 base pair BsmI to BsmI DNA fragment. The final plasmid vector was constructed by ligation and transformation (as described above) of DNA fragments obtained by cutting end isolating a 2 kilo-base pair SstI to BamHI DNA fragment from the intermediete vector, a 2.8 kbp SstI to EcoRI DNA fragment from the plasmid vector, and a 360 bp BamHI to EcoRI DNA fragment from the

plasmid vector. The final construct was verified by DNA sequencing the G-CSF gene. Cultures were grown, and the cells were harvested, and the G-CSF analogs were purified as set forth below.

As indicated above, any combination of mutagenesis techniques may be used to generate a G-CSF analog nucleic acid (and expression product) having one or more than one alteration. The two examples above, using M13-based mutagenesis and gene amplification-based mutagenesis, are illustrative.

E. Expression of G-CSF Analog DNA

The G-CSF analog DNAs were then placed into a plasmid vector and used to transform E. coli strain FM5 (ATCC#53911). The present G-CSF analog DNAs contained on plasmids and in bacterial host cells are available from the American Type Culture Collection, Rockville, MD, and the accession designations are indicated below.

One liter cultures were grown in broth containing 10g tryptone, 5g yeast extract and 5g NaCl) at 30°C until reaching a density at A^{600} of 0.5, at which point they were rapidly heated to 42°C. The flasks were allowed to continue shaking at for three hours.

Other prokaryotic or eukaryotic host cells may also be used, such as other bacterial cells, strains or species, mammalian cells in culture (COS, CHO or other types) insect cells or multicellular organs or organisms, or plant cells or multicellular organs or organisms, and a skilled practitioner will recognize the appropriate host. The present G-CSF analogs and related compositions may also be prepared synthetically, as, for example, by solid phase peptide synthesis methds, or other chemical manufacturing techniques. Other cloning and expression systems will be apparent to those skilled in the art.

F. Purification of G-CSF Analog Protein

Cells were harvested by centrifugation (10,000 x G, 20 minutes, 4°C). The pellet (usually 5 grams) was resuspended in 30 ml of 1mM DTT and passed three times through a French press cell at 10,000 psi. The broken cell suspension was centrifuged at 10,000g for 30 minutes, the supernatant removed, and the pellet resuspended in 30-40 ml water. This was recentrifuged at 10,000 x G for 30 minutes, and this pellet was dissolved in 25 ml of 2% Sarkosyl and 50mM Tris at pH 8. Copper sulfate was added to a concentration of 40uM, and the mixture was allowed to stir for at least 15 hours at 15-25°C. The mixture was then centrifuged at 20,000 x G for 30 minutes. The resultant solubilized protein mixture was diluted four-fold with 13.3 mM Tris, pH 7.7, the Sarkosyl was removed, and the supernatant was then applied to a DEAE-cellulose (Whatman DE-52) column equilibrated in 20mM Tris, pH 7.7. After loading and washing the column with the same buffer, the analogs were eluted with 20mM Tris /NaCl (between 35mM to 100mM depending on the analog, as indicated below), pH 7.7. For most of the analogs, the eluent from the DEAE column was adjusted to a pH of 5.4, with 50% acetic acid and diluted as necessary (to obtain the proper conductivity) with 5mM sodium acetate pH 5.4. The solution was then loaded onto a CM-sepharose column equilibrated in 20 mM sodium acetate, pH 5.4. The column was then washed with 20mM NaAc, pH 5.4 until the absorbance at 280 nm was approximately zero. The G-CSF analog was then eluted with sodium acetate/NaCl in concentrations as described below in Table 4. The DEAE column eluents for those analogs not applied to the CM-sepharose column were dialyzed directly into 10mM NaAc, ph 4.0 buffer. The purified G-CSF analogs were then suitably isolated for in vitro analysis. The salt concentrations used for eluting the analogs varied, as noted above. Below, the salt concentrations for the DEAE cellulose column and for the CM-sepharose column are listed:

Table 4
Salt Concentrations

Analog	DEAE Cellulose	CM-Sepharose
Lys^{17}->Arg^{17}	35mM	37.5mM
Lys^{24}->Arg^{24}	35mM	37.5mM
Lys^{35}->Arg^{35}	35mM	37.5mM
Lys^{41}->Arg^{41}	35mM	37.5mM
$Lys^{17,24,35}$->$Arg^{17,24,35}$	35mM	37.5mM
$Lys^{17,35,41}$->$Arg^{17,35,41}$	35mM	37.5mM

Table 4 Con't

Analog	DEAE Cellulose	CM-Sepharose
$Lys^{24,35,41}$->$Arg^{24,35,41}$	35mM	37.5mM
$Lys^{17,24,35,41}$->$Arg^{17,24,35,41}$	35mM	37.5mM
$Lys^{17,24,41}$->$Arg^{17,24,41}$	35mM	37.5mM
Gln^{68}->Glu^{68}	60mM	37,5mM
$Cys^{37,43}$->$Ser^{37,43}$	40mM	37.5mM
Gln^{26}->Ala^{26}	40mM	40mM
Gln^{174}->Ala^{174}	40mM	40mM
Arg^{170}->Ala^{170}	40mM	40mM
Arg^{167}->Ala^{167}	40mM	40mM
Deletion 167*	N/A	N/A
Lys^{41}->Ala^{41}	160mM	40mM
His^{44}->Lys^{44}	40mM	60mM
Glu^{47}->Ala^{47}	40mM	40mM
Arg^{23}->Ala^{23}	40mM	40mM
Lys^{24}->Ala^{24}	120mM	40mM
Glu^{20}->Ala^{20}	40mM	60mM
Asp^{28}->Ala^{28}	40mM	80mM
Met^{127}->Glu^{127}	80mM	40mM
Met^{138}->Glu^{138}	80mM	40mM
Met^{127}->Leu^{127}	40mM	40mM
Met^{138}->Leu^{138}	40mM	40mM
Cys^{18}->Ala^{18}	40mM	37.5mM
$Gln^{12,21}$->$Glu^{12,21}$	60mM	37.5mM
$Gln^{12,21,68}$->$Glu^{12,21,68}$ Glu^{20}->Ala^{20}; Ser^{13}->Gly^{13}	60mM	37.5mM
	40mM	80mM

Table 4 Con't

Analog	DEAE Cellulose	CM-Sepharose
$Met^{127,138}$->$Leu^{127,138}$	40mM	40mM
Ser^{13}->Ala^{13}	40mM	40mM
Lys^{17}->Ala^{17}	80mM	40mM
Gln^{121}->Ala^{121}	40mM	60mM
Gln^{21}->Ala^{21}	50mM	Gradient 0 -150mM
His^{44}->Ala^{44}**	40mM	N/A
His^{53}->Ala^{53}**	50mM	N/A
Asp^{110}->Ala^{110}**	40mM	N/A
Asp^{113}->Ala^{113}**	40mM	N/A
Thr^{117}->Ala^{117}**	50mM	N/A
Asp^{28}->Ala^{28}; Asp^{110} Ala^{110}**	50mM	N/A
Glu^{124}->Ala^{124}**	40mM	40mM

* For Deletion ^{167}, the data are unavailable.
** For these analogs, the DEAE cellulose column alone was use for purification.

The above purification methods are illustrative, and a skilled practitioner will recognize that other means are available for obtaining the present G-CSF analogs.

G. Biological Assays

Regardless of which methods were used to create the present G-CSF analogs, the analogs were subject to assays for biological activity. Tritiated thymidine assays were conducted to ascertain the degree of cell division. Other biological assays, however, may be used to ascertain the desired activity. Biological assays such as assaying for the ability to induce terminal differentiation in mouse WEHI-3B (D+) leukemic cell line, also provides indication of G-CSF activity. See Nicola, et al., Blood 54: 614-27 (1979). Other in vitro assays may be used to ascertain biological activity. See Nicola, Annu. Rev. Biochem. 58: 45-77 (1989). In general, the test for biological activity should provide analysis for the desired result, such as increase or decrease in biological activity (as compared to non-altered G-CSF), different biological activity (as compared to non-altered G-CSF), receptor affinity analysis, or serum half-life analysis. The list is incomplete, and those skilled in the art will recognize other assays useful for testing for the desired end result.

The ^{3}H-thymidine assay was performed using standard methods. Bone marrow was obtained from sacrificed female Balb C mice. Bone marrow cells were briefly suspended, centrifuged, and resuspended in a growth medium. A 160 ul aliquot containing approximately 10,000 cells was placed into each well of a 96 well micro-titer plate. Samples of the purified G-CSF analog(as prepared above) were added to each well, and incubated for 68 hours. Tritiated thymidine was added to the wells and allowed to incubate for 5 additional hours. After the 5 hour incubation time, the cells were harvested, filtered, and thoroughly rinsed. The filters were added to a vial containing scintillation fluid. The beta emissions were counted (LKB Betaplate scintillation counter). Standards and analogs were analyzed in triplicate, and samples which fell substantially above or below the standard curve were re-assayed with the proper dilution. The results

reported here are the average of the triplicate analog data relative to the unaltered recombinant human G-CSF standard results.

H. HPLC Analysis

High pressure liquid chromatography was performed on purified samples of analog. Although peak position on a reverse phase HPLC column is not a definitive indication of structural similarity between two proteins, analogs which have similar retention times may have the same type of hydrophobic interactions with the HPLC column as the non-altered molecule. This is one indication of an overall similar structure.

Samples of the analog and the non-altered recombinant human G-CSF were analyzed on a reverse phase (0.46 x 25 cm) Vydac 214TP54 column (Separations Group, Inc. Hesperia, CA). The purified analog G-CSF samples were prepared in 20 mM acetate and 40 mM NaCl solution buffered at pH 5.2 to a final concentration of 0.1 mg/ml to 5 mg/ml, depending on how the analog performed in the column. Varying amounts (depending on the concentration) were loaded onto the HPLC column, which had been equilibrated with an aqueous solution containing 1% isopropanol, 52.8% acetonitrile, and .38% trifluoro acetate (TFA). The samples were subjected to a gradient of 0.86%/minute acetonitrile, and .002% TFA.

I. Results

Presented below are the results of the above biological assays and HPLC analysis. Biological activity is the average of triplicate data and reported as a percentage of the control standard (non-altered G-CSF). Relative HPLC peak position is the position of the analog G-CSF relative to the control standard (non-altered G-CSF) peak. The "+" or "-" symbols indicate whether the analog HPLC peak was in advance of or followed the control standard peak (in minutes). Not all of the variants had been analyzed for relative HPLC peak, and only those so analyzed are included below. Also presented are the American Type Culture Collection designations for E. coli host cells containing the nucleic acids coding for the present analogs, as prepared above.

Table 5

Seq. ID	Variant	Analog	Relative HPLC Peak	ATCC No.	% Normal G-CSF Activity
67	1	Lys^{17}->Arg^{17}	N/A	69184	N/A
68	2	Lys^{24}->Arg^{24}	N/A	69185	N/A
69	3	Lys^{35}->Arg^{35}	N/A	69186	N/A
70	4	Lys^{41}->Arg^{41}	N/A	69187	N/A
71	5	$Lys^{17,24,35}$->$Arg^{17,24,35}$	N/A	69189	N/A
72	6	$Lys^{17,35,41}$->$Arg^{17,35,41}$	N/A	69192	N/A
73	7	$Lys^{24,35,41}$->$Arg^{24,35,41}$	N/A	69191	N/A
74	8	$Lys^{17,24,35,41}$ ->$Arg^{17,24,35,41}$	N/A	69193	N/A
75	9	$Lys^{17,24,41}$->$Arg^{17,24,41}$	N/A	69190	N/A
76	10	Gln^{68}->Glu^{68}	N/A	69196	N/A
77	11	$Cys^{37,43}$->$Ser^{37,43}$	N/A	69197	N/A
78	12	Gln^{26}->Ala^{26}	+.96	69201	51%
79	13	Gln^{174}->Ala^{174}	+.14	69202	100%
80	14	Arg^{170}->Ala^{170}	+.78	69203	100%

Table 5 Con't

Seq. ID	Variant	Analog	Relative HPLC Peak	ATCC No.	% Normal G-CSF Activity
81	15	Arg^{167}->Ala^{167}	+.54	69204	110%
82	16	Deletion 167	-.99	69207	N/A
83	17	Lys^{41}->Ala^{41}	+.25	69208	81%
84	18	His^{44}->Lys^{44}	-1.53	69212	70%
85	19	Glu^{47}->Ala^{47}	+.14	69205	0%
86	20	Arg^{23}->Ala^{23}	-.03	69206	31%
87	21	Lys^{24}->Ala^{24}	+1.95	69213	0%
88	22	Glu^{20}->Ala^{20}	-0.07	69211	0%
89	23	Asp^{28}->Ala^{28}	-.30	69210	147%
90	24	Met^{127}->Glu^{127}	N/A	69223	N/A
91	25	Met^{138}->Glu^{138}	N/A	69222	N/A
92	26	Met^{127}->Leu^{127}	N/A	69198	N/A
93	27	Met^{138}->Leu^{138}	N/A	69199	N/A
94	28	Cys^{18}->Ala^{18}	N/A	69188	N/A
95	29	$Gln^{12,21}$->$Glu^{12,21}$	N/A	69194	N/A
96	30	$Gln^{12,21,68}$->$Glu^{12,21,68}$	N/A	69195	N/A
97	31	Glu^{20}->Ala^{20}; Ser^{13}	+1.74	69209	0%

Table 5 Con't

Seq. ID	Variant	Analog	Relative HPLC Peak	ATCC No.	% Normal G-CSF Activity
		->Gly^{13}			
98	32	$Met^{127,138}$->$Leu^{127,138}$	+1.43	69200	98%
99	33	Ser^{13}->Ala^{13}	0	69221	110%
100	34	Lys^{17}->Ala^{17}	+.50	69226	70%
101	35	Gln^{121}->Ala^{121}	+2.7	69225	100%
102	36	Gln^{21}->Ala^{21}	+0.63	69217	9.6%
103	37	His^{44}->Ala^{44}	+1.52	69215	10.8%
104	38	His^{53}->Ala^{53}	+0.99	69219	8.3%
105	39	Asp^{110}->Ala^{110}	+1.97	69216	29%
106	40	Asp^{113}->Ala^{113}	-0.34	69218	0%
107	41	Thr^{117}->Ala^{117}	+0.4	69214	9.7%
108	42	Asp^{28}->Ala^{28}; Asp^{110} Ala^{110}	+3.2	69220	20.6%

Table 5 Con't

Seq. ID	Variant	Analog	Relative HPLC Peak	ATCC No.	% Normal G-CSF Activity
109	43	Glu124->Ala124	+0.16	69224	75%
110	44	Phe114->Val 114, Thr117->Ala117**	+0.53		0%

**This analog was apparently a result of an inadvertent error in the oligo which was used to prepare number 41, above (Thr117->Ala 117), and thus was prepared identically to the process used for that analog.

N/A* indicates data which are not available.

1. Identification of Structure-Function Relationships

The first step used to design the present analogs was to determine what moieties are necessary for structural integrity of the G-CSF molecule. This was done at the amino acid residue level, although the

atomic level is also available for analysis. Modification of the residues necessary for structural integrity results in change in the overall structure of the G-CSF molecule. This may or may not be desirable, depending on the analog one wishes to produce. The working examples here were designed to maintain the overall structural integrity of the G-CSF molecule, for the purpose of maintain G-CSF receptor binding of the analog to the G-CSF receptor (as used in this section below, the "G-CSF receptor" refers to the natural G-CSF receptor, found on hematopoietic cells). It was assumed, and confirmed by the studies presented here, that G-CSF receptor binding is a necessary step for at least one biological activity, as determined by the above biological assays.

As can be seen from the figures, G-CSF (here, recombinant human met-G-CSF) is an antiparallel 4-alpha helical bundle with a left-handed twist, and with overall dimensions of 45 Å x 30Å x 24Å. The four helices within the bundle are referred to as helices A, B, C and D, and their connecting loops are known as the AB, BC and CD loops. The helix crossing angles range from -167.5° to -159.4°. Helices A, B, and C are straight, whereas helix D contains two kinds of structural characteristics, at Gly 150 and Ser 160 (of the recombinant human met-G-CSF). Overall, the G-CSF molecules is a bundle of four helices, connected in series by external loops. This structural information was then correlated with known functional information. It was known that residues (including methionine at position 1) 47, 23, 24, 20, 21, 44, 53, 113, 110, 28 and 114 may be modified, and the effect on biological activity would be substantial.

The majority of single mutations which lowered biological activity were centered around two regions of G-CSF that are separated by 30Å, and are located on different faces of the four helix bundle. One region involves interactions between the A helix and the D helix. This is further confirmed by the presence of salt bridges in the non-altered molecule as follows:

Atom	Helix	Atom	Helix	Distance
Arg 170 N1	D	Tyr 166 OH	A	3.3
Tyr 166 OH	D	Arg 23 N2	A	3.3
Glu 163 OE1	D	Arg 23 N1	A	2.8
Arg 23 N1	A	Gln 26 OE1	A	3.1
Gln 159 NE2	D	Gln 26 O	A	3.3

Distances reported here were for molecule A, as indicated in FIGURE 5 (wherein three G-CSF molecules crystallized together and were designated as A, B, and C). As can be seen, there is a web of salt bridges between helix A and helix D, which act to stabilize the helix A structure, and therefore affect the overall structure of the G-CSF molecule.

The area centering around residues Glu 20, Arg 23 and Lys 24 are found on the hydrophilic face of the A helix (residues 20-37). Substitution of the residues with the non-charged alanine residue at positions 20 and 23 resulted in similar HPLC retention times, indicating similarity in structure. Alteration of these sites altered the biological activity (as indicated by the present assays). Substitution at Lys 24 altered biological activity, but did not result in a similar HPLC retention time as the other two alterations.

The second site at which alteration lowered biological activity involves the AB helix. Changing glutamine at position 47 to alanine (analog no. 19, above) reduced biological activity (in the thymidine uptake assay) to zero. The AB helix is predominantly hydrophobic, except at the amino and carboxy termini; it contains one turn of a 3^{10} helix. There are two histadines at each termini (His 44 and His 56) and an additional glutamate at residue 46 which has the potential to form a salt bridge to His 44. The fourier transformed infra red spectrographic analysis (FTIR) of the analog suggests this analog is structurally similar to the non-altered recombinant G-CSF molecule. Further testing showed that this analog would not crystallize under the same conditions as the non-altered recombinant molecule.

Alterations at the carboxy terminus (Gln 174, Arg 167 and Arg 170) had little effect on biological activity. In contrast, deletion of the last eight residues (167-175) lowered biological activity. These results may indicate that the deletion destabilizes the overall structure which prevents the mutant from proper binding to the G-CSF receptor (and thus initiating signal transduction).

Generally, for the G-CSF internal core -- the internal four helix bundle lacking the external loops --the hydrophobic internal residues are essential for structural integrity. For example, in helix A, the internal hydrophobic residues are (with methionine being position 1) Phe 14, Cys 18, Val 22, Ile 25, Ile 32 and Leu 36. Generally, for the G-CSF internal core -- the internal four helix bundle lacking the external loops --the hydrophobic internal residues are essential for structural integrity. For example, in helix A, the internal hydrophobic residues are (with methionine being position 1 as in FIGURE 1) Phe 14, Cys 18, Val 22, Ile 25, Ile 32 and Leu 36. The other hydrophobic residues (again with the met at position 1) are: helix B, Ala 72,

Leu 76, Leu 79, Leu 83, Tyr 86, Leu 90 Leu 93; helix C, Leu 104, Leu 107, Val 111, Ala 114, Ile 118, Met 122; and helix D, Val 154, Val 158, Phe 161, Val 164, Val 168, Leu 172.

The above biological activity data, from the presently prepared G-CSF analogs, demonstrate that modification of the external loops interfere least with G-CSF overall structure. Preferred loops for analog prepration are the AB loop and the CD loop. The loops are relatively flexible structures as compared to the helices. The loops may contribute to the proteolysis of the molecule. G-CSF is relatively fast acting in vivo as the purpose the molecule serves is to generate a response to a biological challenge, i.e., selectively stimulate neutrophils. The G-CSF turnover rate is also relatively fast. The flexibility of the loops may provide a "handle" for proteases to attach to the molecule to inactivate the molecule. Modification of the loops to prevent protease degradation, yet have (via retention of the overall structure of non-modified G-CSF) no loss in biological activity may be accomplished.

This phenomenon is probably not limited to the G-CSF molecule but may also be common to the other molecules with known similar overall structures, as presented in Figure 2. Alteration of the external loop of, for example hGH, Interferon B, IL-2, GM-CSF and IL-4 may provide the least change to the overall structure. The external loops on the GM-CSF molecule are not as flexible as those found on the G-CSF molecule, and this may indicate a longer serum life, consistent with the broader biological activity of GM-CSF. Thus, the external loops of GM-CSF may be modified by releasing the external loops from the beta-sheet structure, which may make the loops more flexible (similar to those G-CSF) and therefore make the molecule more susceptible to protease degradation (and thus increase the turnover rate).

Alteration of these external loops may be effected by stabilizing the loops by connection to one or more of the internal helices. Connecting means are known to those in the art, such as the formation of a beta sheet, salt bridge, disulfide bonding or hydrophobic interactions, and other means are available. Also, deletion of one or more moieties, such as one or more amino acid residues or portions thereof, to prepare an abbreviated molecule and thus eliminate certain portions of the external loops may be effected.

Thus, by alteration of the external loops, preferably the AB loop (amino acids 58-72 of r-hu-met G-CSF) or the CD loop (amino acids 119 to 145 of r-hu-met-G-CSF), and less preferably the amino terminus (amino acids 1-10), one may therefore modify the biological function without elimination of G-CSF G-CSF receptor binding. For example, one may: (1) increase half-life (or prepare an oral dosage form, for example) of the G-CSF molecule by, for example, decreasing the ability of proteases to act on the G-CSF molecule or adding chemical modifications to the G-CSF molecule, such as one or more polyethylene glycol molecules or enteric coatings for oral formulation which would act to change some characteristic of the G-CSF molecule as described above, such as increasing serum or other half-life or decreasing antigenicity; (2) prepare a hybrid molecule, such as combining G-CSF with part or all of another protein such as another cytokine or another protein which effects signal transduction via entry through the cell through a G-CSF G-CSF receptor transport mechanism; or (3) increase the biological activity as in, for example, the ability to selectively stimulate neutrophils (as compared to a non-modified G-CSF molecule). This list is not limited to the above exemplars.

Another aspect observed from the above data is that stabilizing surface interactions may affect biological activity. This is apparent from comparing analogs 23 and 40. Analog 23 contains a substitution of the charged asparagine residue at position 28 for the neutrally-charged alanine residue in that position, and such substitution resulted in a 50% increase in the biological activity (as measured by the disclosed thymidine uptake assays). The asparagine residue at position 28 has a surface interaction with the asparagine residue at position 113; both residues being negatively charged, there is a certain amount of instability (due to the repelling of like charged moieties). When, however the asparagine at position 113 is replaced with the neutrally-charged alanine, the biological activity drops to zero (in the present assay system). This indicates that the asparagine at position 113 is critical to biological activity, and elimination of the asparagine at position 28 serves to increase the effect that asparagine at position 113 possesses.

The domains required for G-CSF receptor binding were also determined based on the above analogs prepared and the G-CSF structure. The G-CSF receptor binding domain is located at residues (with methionine being position 1) 11-57 (between the A and AB helix) and 100-118 (between the B and C helices). One may also prepare abbreviated molecules capable of binding to a G-CSF receptor and initiate signal transduction for selectively stimulating neutrophils by changing the external loop structure and having the receptor binding domains remain intact.

Residues essential for biological activity and presumably G-CSF receptor binding or signal transduction have been identified. Two distinct sites are located on two different regions of the secondary structure. What is here called "Site A" is located on a helix which is constrained by salt bridge contacts between two other members of the helical bundle. The second site, "Site B" is located on a relatively more flexible helix, AB. The AB helix is potentially more sensitive to local pH changes because of the type and position of the

residues at the carboxy and amino termini. The functional importance of this flexible helix may be important in a conformationally induced fit when binding to the G-CSF receptor. Additionally, the extended portion of the D helix is also indicated to be a G-CSF receptor binding domain, as ascertained by direct mutational and indirect comparative protein structure analysis. Deletion of the carboxy terminal end of r-hu-met-G-CSF reduces activity as it does for hGH, see, Cunningham and Wells, Science 244: 1081-1084 (1989). Cytokines which have similar structures, such as IL-6 and GM-CSF with predicted similar topology also center their biological activity along the carboxy end of the D helix, see Bazan, Immunology Today 11: 350-354 (1990)

A comparison of the structures and the positions of G-CSF receptor binding determinants between G-CSF and hGH suggests both molecules have similar means of signal transduction. Two separate G-CSF receptor binding sites have been identified for hGH De Vos et al., Science 255: 306-32 (1991). One of these binding sites (called "Site I") is formed by residues on the exposed faces of hGH's helix 1, the connection region between helix 1 and 2, and helix 4. The second binding site (called "Site II") is formed by surface residues of helix 1 and helix 3.

The G-CSF receptor binding determinates identified for G-CSF are located in the same relative positions as those identified for hGH. The G-CSF receptor binding site located in the connecting region between helix A and B on the AB helix (Site A) is similar in position to that reported for a small piece of helix (residues 38-47) of hGH. A single point mutation in the AB helix of G-CSF significantly reduces biological activity (as ascertained in the present assays), indicating the role in a G-CSF receptor-ligand interface. Binding of the G-CSF receptor may destabilize the 3^{10} helical nature of this region and induce a conformation change improving the binding energy of the ligand/G-CSF receptor complex.

In the hGH receptor complex, the first helix of the bundle donates residues to both of the binding sites required to dimerize the hGH receptor Mutational analysis of the corresponding helix of G-CSF (helix A) has identified three residues which are required for biological activity. Of these three residues, Glu 20 and Arg 24 lie on one face of the helical bundle towards helix C, whereas the side chain of Arg 23 (in two of the three molecules in the asymmetric unit) points to the face of the bundle towards helix D. The position of side chains of these biologically important residues indicates that similar to hGH, G-CSF may have a second G-CSF receptor binding site along the interface between helix A and helix C. In contrast with the hGH molecule, the amino terminus of G-CSF has a limited biological role as deletion of the first 11 residues has little effect on the biological activity.

As indicated above (see FIGURE 2, for example), G-CSF has a topological similarity with other cytokines. A correlation of the structure with previous biochemical studies, mutational analysis and direct comparison of specific residues of the hGH receptor complex indicates that G-CSF has two receptor binding sites. Site A lies along the interface of the A and D helices and includes residues in the small AB helix. Site B also includes residues in the A helix but lies along the interface between helices A and C. The conservation of structure and relative positions of biologically important residues between G-CSF and hGH is one indication of a common method of signal transduction in that the receptor is bound in two places. It is therefore found that G-CSF analogs possessing altered G-CSF receptor binding domains may be prepared by alteration at either of the G-CSF receptor binding sites (residues 20-57 and 145-175).

Knowledge of the three dimensional structure and correlation of the composition of G-CSF protein makes possible a systematic, rational method for preparing G-CSF analogs. The above working examples have demonstrated that the limitations of the size and polarity of the side chains within the core of the structure dictate how much change the molecule can tolerate before the overall structure is changed.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Amgen Inc.

(ii) TITLE OF INVENTION: G-CSF ANALOG COMPOSITIONS AND METHODS

(iii) NUMBER OF SEQUENCES: 110

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Amgen Inc.
(B) STREET: Amgen Center, 1840 DeHavilland Drive
(C) CITY: Thousand Oaks
(D) STATE: California
(E) COUNTRY: United States of America
(F) ZIP: 91320-1789

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 565 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 30..554

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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TCTAGAAAAA ACCAAGGAGG TAATAAATA ATG ACT CCA TTA GGT CCT GCT TCT      53
                                Met Thr Pro Leu Gly Pro Ala Ser
                                  1               5

TCT CTG CCG CAA AGC TTT CTG CTG AAA TGT CTG GAA CAG GTT CGT AAA     101
Ser Leu Pro Gln Ser Phe Leu Leu Lys Cys Leu Glu Gln Val Arg Lys
    10                  15                  20

ATC CAG GGT GAC GGT GCT GCA CTG CAA GAA AAA CTG TGC GCT ACT TAC     149
Ile Gln Gly Asp Gly Ala Ala Leu Gln Glu Lys Leu Cys Ala Thr Tyr
 25                  30                  35                  40
```

```
AAA CTG TGC CAT CCG GAA GAG CTG GTA CTG CTG GGT CAT TCT CTT GGG      197
Lys Leu Cys His Pro Glu Glu Leu Val Leu Leu Gly His Ser Leu Gly
                45                  50                  55

ATC CCG TGG GCT CCG CTG TCT TCT TGT CCA TCT CAA GCT CTT CAG CTG      245
Ile Pro Trp Ala Pro Leu Ser Ser Cys Pro Ser Gln Ala Leu Gln Leu
            60                  65                  70

GCT GGT TGT CTG TCT CAA CTG CAT TCT GGT CTG TTC CTG TAT CAG GGT      293
Ala Gly Cys Leu Ser Gln Leu His Ser Gly Leu Phe Leu Tyr Gln Gly
        75                  80                  85

CTT CTG CAA GCT CTG GAA GGT ATC TCT CCG GAA CTG GGT CCG ACT CTG      341
Leu Leu Gln Ala Leu Glu Gly Ile Ser Pro Glu Leu Gly Pro Thr Leu
    90                  95                  100

GAC ACT CTG CAG CTA GAT GTA GCT GAC TTT GCT ACT ACT ATT TGG CAA      389
Asp Thr Leu Gln Leu Asp Val Ala Asp Phe Ala Thr Thr Ile Trp Gln
105                 110                 115                 120

CAG ATG GAA GAG CTC GGT ATG GCA CCA GCT CTG CAA CCG ACT CAA GGT      437
Gln Met Glu Glu Leu Gly Met Ala Pro Ala Leu Gln Pro Thr Gln Gly
                125                 130                 135

GCT ATG CCG GCA TTC GCT TCT GCA TTC CAG CGT CGT GCA GGA GGT GTA      485
Ala Met Pro Ala Phe Ala Ser Ala Phe Gln Arg Arg Ala Gly Gly Val
            140                 145                 150

CTG GTT GCT TCT CAT CTG CAA TCT TTC CTG GAA GTA TCT TAC CGT GTT      533
Leu Val Ala Ser His Leu Gln Ser Phe Leu Glu Val Ser Tyr Arg Val
        155                 160                 165

CTG CGT CAT CTG GCT CAG CCG TAATAGAATT C                             565
Leu Arg His Leu Ala Gln Pro
    170                 175
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45
```

```
Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
 50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CTTTCTGCTG CGTTGTCTGG AACA 24

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ACAGGTTCGT CGTATCCAGG GTG 23

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CACTGCAAGA ACGTCTGTGC GCT 23

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CGCTACTTAC CGTCTGTGCC ATC 23

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CTTTCTGCTG CGTTGTCTGG AACA 24

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ACAGGTTCGT CGTATCCAGG GTG 23

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CACTGCAAGA ACGTCTGTGC GCT 23

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CTTTCTGCTG CGTTGTCTGG AACA 24

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ACAGGTTCGT CGTATCCAGG GTG 23

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CGCTACTTAC CGTCTGTCCC ATC 23

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CTTTCTGCTG CGTTGTCTGG AACA 24

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CACTGCAAGA ACGTCTGTGC GCT 23

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CGCTACTTAC CGTCTGTGCC ATC 23

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

ACAGGTTCGT CGTATCCAGG GTG 23

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CACTGCAAGA ACGTCTGTGC GCT 23

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CGCTACTTAC CGTCTGTGCC ATC 23

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CTTTCTGCTG CGTTGTCTGG AACA 24

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

ACAGGTTCGT CGTATCCAGG GTG 23

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CACTGCAAGA ACGTCTGTGC GCT 23

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CGCTACTTAC CGTCTGTGCC ATC 23

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TCTGCTGAAA GCTCTGGAAC AGG 23

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CTTGTCCATC TGAAGCTCTT CAG 23

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GAAAAACTGT CCGCTACTTA CAAACTGTCC CATCCGG 37

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

TTCGTAAAAT CGCGGGTGAC GG 22

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

TCATCTGGCT GCGCCGTAAT AG 22

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CCGTGTTCTG GCTCATCTGG CT 22

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GAAGTATCTT ACGCTGTTCT GCGT 24

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GAAGTATCTT ACTAAGTTCT GCGTC 25

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CGCTACTTAC GCACTGTGCC AT 22

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CAAACTGTGC AAGCCGGAAG AG 22

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CATCCGGAAG CACTGGTACT GC 22

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GGAACAGGTT GCTAAAATCC AGG 23

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GAACAGGTTC GTGCGATCCA GGGTG 25

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GAAATGTCTG GCACAGGTTC GT 22

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

TCCAGGGTGC CGGTGCTGC 19

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

AAGAGCTCGG TGAGGCACCA GCT 23

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

CTCAAGGTGC TGAGCCGGCA TTC 23

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GAGCTCGGTC TGGCACCAGC 20

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

TCAAGGTGCT CTGCCGGCAT T 21

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

TCTGCCGCAA GCCTTTCTGC TGA 23

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

CTTTCTGCTG GCATGTCTGG AACA 24

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

CTATTTGGCA AGCGATGGAA GAGC 24

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

CAGATGGAAG CGCTCGGTAT G 21

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

GAGCTCGGTC TGGCACCAGC 20

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

TCAAGGTGCT CTGCCGGCAT T 21

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

GAAATGTCTG GCACAGGTTC GT 22

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

TTCCGGAGCG CACAGTTTG 19

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

CGAGAAGGCC TCGGGTGTCA AAC 23

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

ATGCCAAATT GCAGTAGCAA AG 22

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

ACAACGGTTT AACGTCATCG TTTC 24

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

ATCAGCTACT GCTAGCTGCA GA 22

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

TCAGTCGATG ACGATCGACG TCT 23

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

TTACGAACCG CTTCCAGACA TT 22

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

TAAAATGCTT GGCGAAGGTC TGTAA 25

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

GTAGCAAATG CAGCTACATC TA 22

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

CATCATCGTT TACGTCGATG TAGAT 25

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

CCAAGAGAAG CACCCAGCAG 20

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

AGGGTTCTCT TCGTGGGTCG TC 22

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

CACTGGCGGT GATAATGAGC 20

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

CTAGGCCAGG CATTACTGG 19

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

CCACTGGCGG TGATACTGAG C 21

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

AGCAGAAAGC TTTCCGGCAG AGAAGAAGCA GGA 33

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 54 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

GCCGCAAAGC TTTCTGCTGA AATGTCTGGA AGAGGTTCGT AAAATCCAGG GTGA 54

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 59 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

CTGGAATGCA GAAGCAAATG CCGGCATAGC ACCTTCAGTC GGTTGCAGAG CTGGTGCCA 59

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Arg Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Glu Gln Val Arg Arg Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30
```

```
Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
          35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
      50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
  65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                  85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
             100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
         115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
     130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                 165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
  1               5                  10                  15

Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
             20                  25                  30

Gln Glu Arg Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
         35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
     50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
 65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                 85                  90                  95
```

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
100 105 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
115 120 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
130 135 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145 150 155 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
165 170 175

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
1 5 10 15

Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
20 25 30

Gln Glu Lys Leu Cys Ala Thr Tyr Arg Leu Cys His Pro Glu Glu Leu
35 40 45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
50 55 60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65 70 75 80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
85 90 95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
100 105 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
115 120 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
130 135 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145 150 155 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
165 170 175

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
1 5 10 15

Arg Cys Leu Glu Gln Val Arg Arg Ile Gln Gly Asp Gly Ala Ala Leu
20 25 30

Gln Glu Arg Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
35 40 45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
50 55 60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65 70 75 80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
85 90 95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
100 105 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
115 120 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
130 135 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145 150 155 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
165 170 175

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Arg Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Arg Leu Cys Ala Thr Tyr Arg Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Glu Gln Val Arg Arg Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Arg Leu Cys Ala Thr Tyr Arg Leu Cys His Pro Glu Glu Leu
        35                  40                  45
```

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
50 55 60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65 70 75 80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
85 90 95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
100 105 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
115 120 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
130 135 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145 150 155 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
165 170 175

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
1 5 10 15

Arg Cys Leu Glu Gln Val Arg Arg Ile Gln Gly Asp Gly Ala Ala Leu
20 25 30

Gln Glu Arg Leu Cys Ala Thr Tyr Arg Leu Cys His Pro Glu Glu Leu
35 40 45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
50 55 60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65 70 75 80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
85 90 95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
100 105 110

```
Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
          115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
      130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                    165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
  1               5                  10                  15

Arg Cys Leu Glu Gln Val Arg Arg Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Arg Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15
Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60

Cys Pro Ser Glu Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Ser Ala Thr Tyr Lys Leu Ser His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Glu Gln Val Arg Lys Ile Ala Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60
```

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65 70 75 80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
85 90 95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
100 105 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
115 120 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
130 135 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145 150 155 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
165 170 175

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
1 5 10 15

Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
20 25 30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
35 40 45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
50 55 60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65 70 75 80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
85 90 95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
100 105 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
115 120 125

```
Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Ala Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Ala His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Ala Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 174 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Val Leu Arg His Leu Ala Gln Pro
                165                 170                 174
```

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Ala Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60
```

```
Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
 65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys Lys Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125
```

```
Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Ala Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Glu Gln Val Ala Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Glu Gln Val Arg Ala Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Ala Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60
```

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65 70 75 80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
85 90 95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
100 105 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
115 120 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
130 135 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145 150 155 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
165 170 175

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
1 5 10 15

Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Ala Gly Ala Ala Leu
20 25 30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
35 40 45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
50 55 60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65 70 75 80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
85 90 95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
100 105 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
115 120 125

```
Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
  1               5                  10                  15

Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Glu Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Glu Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Leu Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60
```

```
Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
 65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Leu Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:94:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Lys Ala Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125
```

```
Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:95:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Glu Ser Phe Leu Leu
  1               5                  10                  15

Lys Cys Leu Glu Glu Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:96:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Glu Ser Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Glu Glu Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60

Cys Pro Ser Glu Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:97:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Gly Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Ala Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:98:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45
```

```
Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
     50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
 65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                 85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Leu Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Leu Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:99:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ala Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110
```

```
Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:100:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
  1               5                  10                  15

Ala Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:101:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Ala Met Glu Glu Leu Gly Met Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:102:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Glu Ala Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:103:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys Ala Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60
```

```
Cys Pro Ser Gln Ala L u Gln Leu Ala Gly Cys Leu Ser Gln Leu His
 65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                 85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:104:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly Ala Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
 65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                 85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125
```

```
Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:105:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Ala Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:106:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Ala Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:107:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:

```
Met Thr Pro Leu Gly Pro Ala S r Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Phe Ala Thr Ala Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:108:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Ala Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60
```

```
Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
 65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Ala Val Ala
            100                 105                 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:109:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110
```

```
Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Ala Leu Gly Met Ala
          115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
      130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                  165                 170                 175
```

(2) INFORMATION FOR SEQ ID NO:110:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:

```
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
 1               5                  10                  15

Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
            20                  25                  30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu
        35                  40                  45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
    50                  55                  60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65                  70                  75                  80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
                85                  90                  95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
            100                 105                 110

Asp Val Ala Thr Ala Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
        115                 120                 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
    130                 135                 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145                 150                 155                 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                165                 170                 175
```

Claims

1. A method for preparing a G-CSF analog comprising the steps of:
   (a) viewing information conveying the three dimensional structure of a G-CSF molecule;

(b) selecting from said viewed information at least one site on said G-CSF molecule for alteration;
(c) preparing a G-CSF molecule having such alteration; and
(d) optionally, testing such G-CSF molecule for a desired characteristic.

2. A computer based method for preparing a G-CSF analog comprising the steps of:
(a) providing computer expression of the three dimensional structure of a G-CSF molecule;
(b) selecting from said computer expression at least one site on said G-CSF molecule for alteration;
(c) preparing a G-CSF molecule having such alteration; and,
(d) optionally, testing such G-CSF molecule for a desired characteristic.

3. A method for preparing a G-CSF analog with the aid of a computer comprising:
(a) providing said computer with the means for displaying the three dimensional structure of a G-CSF molecule including displaying the composition of moieties of said G-CSF molecule, preferably displaying the three dimensional location of each amino acid, and more preferably displaying the three dimensional location of each atom of a G-CSF molecule;
(b) viewing said display;
(c) selecting a site on said display for alteration in the composition of said molecule or the location of a moiety; and
(d) preparing a G-CSF analog with such alteration.

4. A computer-based method for preparing a G-CSF analog comprising the steps of:
(a) viewing the three dimensional structure of a G-CSF molecule via a computer, said computer having been previously programmed (i) to express the coordinates of a G-CSF molecule in three dimensional space, and (ii) to allow for entry of information for alteration of said G-CSF expression and viewing thereof;
(b) selecting a site on said visual image of said G-CSF molecule for alteration;
(c) entering information for said alteration on said computer;
(d) viewing a three dimensional structure of said altered G-CSF molecule via said computer;
(e) optionally repeating steps (a)-(e) above;
(f) preparing a G-CSF analog with said alteration; and
(g) optionally testing said G-CSF analog for a desired characteristic.

5. In a computer-based apparatus for displaying the three dimensional structure of a molecule, the improvement comprising means for correlating said three dimensional structure of a G-CSF molecule with the composition of said G-CSF molecule.

6. A method for crystallization of a protein comprising the steps of:
(a) combining, optionally by automated means, aqueous aliquots of said protein with either (i) aliquots of a salt solution, each aliquot having a different concentration of salt; or (ii) aliquots of a precipitant solution, each aliquot having a different concentration of precipitant;
(b) selecting at least one of said combined aliquots, said selection based on the formation of precrystalline forms, or, if no precrystalline forms are so produced, increasing the protein starting concentration of said aqueous aliquots of protein and repeating step (a);
(c) after said salt or said precipitant concentration is selected, repeating step (a) with said previously unselected solution in the presence of said selected concentration; and,
(d) repeating step (b) and step (a) until a crystal of desired quality is obtained.

7. A method of claim 6 wherein each combination pursuant to step (a) is performed in a range of pH.

8. A method of claim 6 wherein said combining of step (a) is done in the presence of a nucleation initiation unit.

9. A G-CSF analog having an amino acid sequence different from that of Figure 1 in that:
(a) the N-terminal methionine is optional; and
(b) one or more of amino acids 58-72 (i) is substituted with one or more different amino acids or (ii) deleted; or (iii) chemically modified.

10. A G-CSF analog of claim 9 wherein said analog is more resistant to proteolysis than a G-CSF molecule of Figure 1.

11. A G-CSF analog of claim 10 wherein at least one of said amino acids is chemically modified by the addition of a polyethylene glycol molecule.

12. A G-CSF analog having an amino acid sequence different from that of Figure 1 in that:
(a) the N-terminal methionine is optional; and
(b) one or more of amino acids 119-125 (i) is substituted with one or more different amino acids or (ii) deleted; or (iii) chemically modified.

13. A G-CSF analog of claim 12 wherein said analog is more resistant to proteolysis than a G-CSF molecule of Figure 1.

14. A G-CSF analog of claim 12 wherein at least one of said amino acids is chemically modified by the addition of a polyethylene glycol molecule.

15. A G-CSF molecule having the AB loop stabilized by connecting such loop to one or more of helices A, B, C, or D.

16. A G-CSF molecule having the CD loop stabilized by connecting such loop to one or more of helices A, B, C, or D.

17. A G-CSF analog, optionally in a pharmaceutically effective carrier, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Lys^{17}->Arg^{17} and the N-terminal methionine is optional.

18. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Lys^{35}->Arg^{35} and the N-terminal methionine is optional.

19. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Lys^{41}->Arg^{41} and the N-terminal methionine is optional.

20. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that $Lys^{17,24,35}$->$Arg^{17,24,35}$ and the N-terminal methionine is optional.

21. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that $Lys^{17,35,41}$->$Arg^{17,35,41}$ and the N-terminal methionine is optional.

22. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that $Lys^{24,35,41}$->$Arg^{24,35,41}$ and the N-terminal methionine is optional.

23. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that $Lys^{17,24,35,41}$->$Arg^{17,24,35,41}$ and the N-terminal methionine is optional.

24. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that $Lys^{17,24,41}$->$Arg^{17,24,41}$ and the N-terminal methionine is optional.

25. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Gln^{68}->Glu^{68} and the N-terminal methionine is optional.

26. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that $Cys^{37,43}$->$Ser^{37,43}$ and the N-terminal methionine is optional.

27. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Gln^{26}->Ala^{26} and the N-terminal methionine is optional.

28. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Gln^{174}->Ala^{174} and the N-terminal methionine is optional.

29. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Arg^{170}->Ala^{170} and the N-terminal methionine is optional.

30. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Arg^{167}->Ala^{167} and the N-terminal methionine is optional.

31. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that there is a deletion at position 167 and the N-terminal methionine is optional.

32. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Lys^{41}->Ala^{41} and the N-terminal methionine is optional.

33. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that His^{44}->Lys^{44} and the N-terminal methionine is optional.

34. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Glu^{47}->Ala^{47} and the N-terminal methionine is optional.

35. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Arg^{23}->Ala^{23} and the N-terminal methionine is optional.

36. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Lys^{24}->Ala^{24} and the N-terminal methionine is optional.

37. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Glu^{20}->Ala^{20} and the N-terminal methionine is optional.

38. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Asp^{28}->Ala^{28} and the N-terminal methionine is optional.

39. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Met^{127}->Glu^{127} and the N-terminal methionine is optional.

40. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from tha of Figure 1 in that Met^{138}->Glu^{138} and the N-terminal methionine is optional.

41. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Met^{127}->Leu^{127} and the N-terminal methionine is optional.

42. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Met^{138}->Leu^{138} and the N-terminal methionine is optional.

43. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Cys^{18}->Ala^{18} and the N-terminal methionine is optional.

44. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that $Gln^{12,21}$->$Glu^{12,21}$ and the N-terminal methionine is optional.

45. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that $Gln^{12,21,68}$->$Glu^{12,21,68}$ and the N-terminal methionine is optional.

46. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Glu^{20}->Ala^{20}; Ser^{13}->Gly^{13} and the N-terminal methionine is optional.

47. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that $Met^{127,138}$->$Leu^{127,138}$ and the N-terminal methionine is optional.

48. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Ser^{13}->Ala^{13} and the N-terminal methionine is optional.

49. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Lys^{17}->Ala^{17} and the N-terminal methionine is optional.

50. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Gln^{121}->Ala^{121} and the N-terminal methionine is optional.

51. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Gln^{21}->Ala^{21} and the N-terminal methionine is optional.

52. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that His^{44}->Ala^{44} and the N-terminal methionine is optional.

53. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein said amino acid sequenc differs from that of Figure 1 in that His^{53}->Ala^{53} and the N-terminal methionine is optional.

54. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Asp^{110}->Ala^{110} and the N-terminal methionine is optional.

55. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Asp^{113}->Ala^{113} and the N-terminal methionine is optional.

56. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Thr^{117}->Ala^{117} and the N-terminal methionine is optional.

57. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Asp^{28}->Ala^{28}; Asp^{110} ->Ala^{110} and the N-terminal methionine is optional.

58. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Glu^{124} ->Ala^{124} and the N-terminal methionine is optional.

59. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Phe^{114}->Val^{114}, Thr^{117}->A^{117} and the N-terminal methionine is optional.

60. The G-CSF analog DNA-containing plasmids and bacterial host cells transformed therewith available from the American Type Culture Collection under the accession numbers ATCC 69184, 69185, 69186, 69187, 69188, 69189, 69190, 69191, 69192, 69193, 69194, 69195, 69196, 69197, 69198, 69199, 69200, 69201, 69202, 69203, 69204, 69205, 69206, 69207, 69208, 69209, 69210, 69211, 69212, 69213, 69214, 69215, 69216, 69217, 69218, 69219, 69220, 69221, 69222, 69223, 69224, 69225 and 69226.

```
                                                  Met Thr Pro Leu Gly Pro Ala
TCTAGAAAAAAACCAAGGAGGTAATAAATA ATG ACT CCA TTA GGT CCT GCT

Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys Cys Leu Glu Gln
TCT TCT CTG CCG CAA AGC TTT CTG CTG AAA TGT CTG GAA CAG

Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln Glu Lys Leu
GTT CGT AAA ATC CAG GGT GAC GGT GCT GCA CTG CAA GAA AAA CTG

Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val Leu Leu
TGC GCT ACT TAC AAA CTG TGC CAT CCG GAA GAG CTG GTA CTG CTG

Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys Pro
GGT CAT TCT CTT GGG ATC CCG TGG GCT CCG CTG TCT TCT TGT CCA

Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser
TCT CAA GCT CTT CAG CTG GCT GGT TGT CTG TCT CAA CTG CAT TCT

Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
GGT CTG TTC CTG TAT CAG GGT CTT CTG CAA GCT CTG GAA GGT ATC

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val
TCT CCG GAA CTG GGT CCG ACT CTG GAC ACT CTG CAG CTA GAT GTA

Ala Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly
GCT GAC TTT GCT ACT ACT ATT TGG CAA CAG ATG GAA GAG CTC GGT

Met Ala Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe
ATG GCA CCA GCT CTG CAA CCG ACT CAA GGT GCT ATG CCG GCA TTC

Ala Ser Ala Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser
GCT TCT GCA TTC CAG CGT CGT GCA GGA GGT GTA CTG GTT GCT TCT

His Leu Gln Ser Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His
CAT CTG CAA TCT TTC CTG GAA GTA TCT TAC CGT GTT CTG CGT CAT

Leu Ala Gln Pro OC  AM
CTG GCT CAG CCG TAA TAG AATTC
```

FIGURE 1

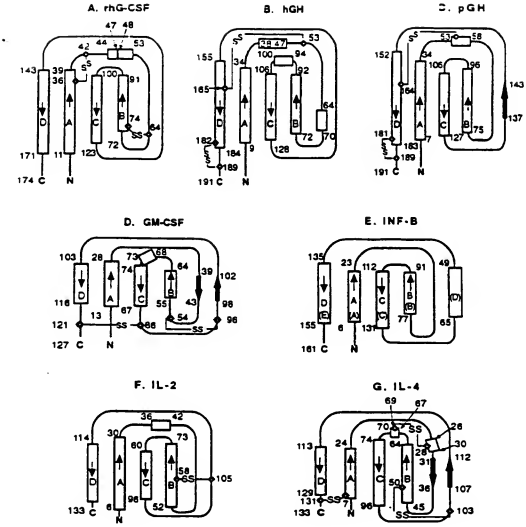

FIGURE 2

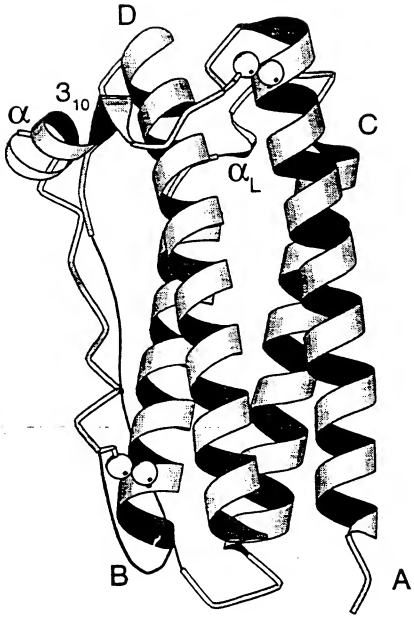

FIGURE 3

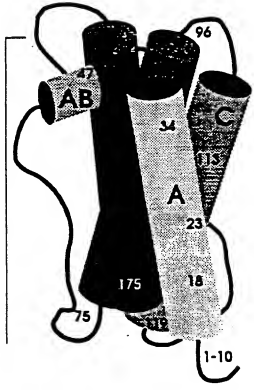

FIGURE 4

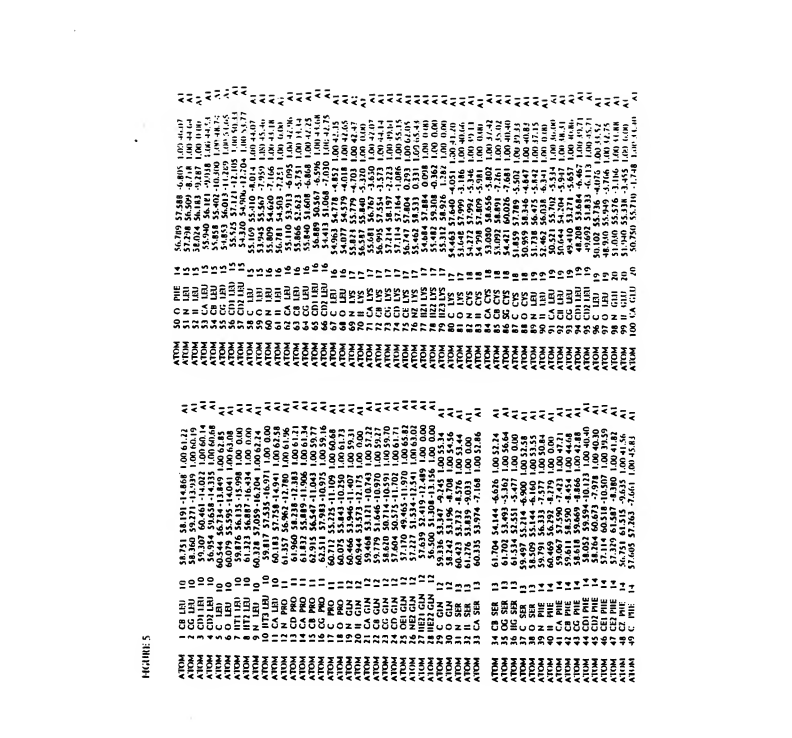

FIGURE 5

ATOM	101	CB	GLU	20	52.053	55.334	-1.167	1.00	35.25	A1
ATOM	102	CG	GLU	20	52.508	55.504	0.260	1.00	43.21	A1
ATOM	103	CD	GLU	20	53.948	54.947	0.407	1.00	51.06	A1
ATOM	104	OE1	GLU	20	54.320	54.660	1.546	1.00	56.78	A1
ATOM	105	OE2	GLU	20	54.708	54.766	-0.570	1.00	51.57	A1
ATOM	106	C	GLU	20	50.230	57.117	-1.326	1.00	33.25	A1
ATOM	107	O	GLU	20	49.432	57.291	-0.380	1.00	33.30	A1
ATOM	108	N	GLN	21	50.660	58.167	-2.044	1.00	32.33	A1
ATOM	109	H	GLN	21	51.270	58.004	-2.794	1.00	0.00	A1
ATOM	110	CA	GLN	21	50.275	59.538	-1.742	1.00	31.00	A1
ATOM	111	CB	GLN	21	51.326	60.489	-2.340	1.00	32.37	A1
ATOM	112	CG	GLN	21	52.436	60.530	-1.272	1.00	38.01	A1
ATOM	113	CD	GLN	21	53.622	61.460	-1.504	1.00	42.67	A1
ATOM	114	OE1	GLN	21	54.008	62.236	-0.615	1.00	43.63	A1
ATOM	115	NE2	GLN	21	54.256	61.448	-2.678	1.00	42.31	A1
ATOM	116	HE21	GLN	21	53.965	60.840	-3.384	1.00	0.00	A1
ATOM	117	HE22	GLN	21	55.026	62.052	-2.730	1.00	0.00	A1
ATOM	118	C	GLN	21	48.894	59.765	-2.288	1.00	28.51	A1
ATOM	119	O	GLN	21	48.027	60.242	-1.563	1.00	28.65	A1
ATOM	120	N	VAL	22	48.682	59.319	-3.521	1.00	25.85	A1
ATOM	121	H	VAL	22	49.448	58.980	-4.013	1.00	0.00	A1
ATOM	122	CA	VAL	22	47.382	59.303	-4.161	1.00	24.94	A1
ATOM	123	CB	VAL	22	47.508	58.614	-5.526	1.00	24.09	A1
ATOM	124	CG1	VAL	22	46.154	58.378	-6.096	1.00	19.97	A1
ATOM	125	CG2	VAL	22	48.252	59.479	-6.498	1.00	25.82	A1
ATOM	126	C	VAL	22	46.418	58.549	-3.226	1.00	25.65	A1
ATOM	127	O	VAL	22	45.428	59.190	-2.800	1.00	29.31	A1
ATOM	128	N	ARG	23	46.643	57.291	-2.759	1.00	23.93	A1
ATOM	129	H	ARG	23	47.440	56.819	-3.056	1.00	0.00	A1
ATOM	130	CA	ARG	23	45.667	56.593	-1.892	1.00	20.67	A1
ATOM	131	CB	ARG	23	46.104	55.135	-1.635	1.00	20.45	A1
ATOM	132	CG	ARG	23	46.325	54.321	-2.904	1.00	17.51	A1
ATOM	133	CD	ARG	23	45.095	54.446	-3.769	1.00	21.54	A1
ATOM	134	NE	ARG	23	45.076	53.437	-4.809	1.00	24.82	A1
ATOM	135	HE	ARG	23	45.642	52.647	-4.701	1.00	0.00	A1
ATOM	136	CZ	ARG	23	44.323	53.556	-5.904	1.00	27.69	A1
ATOM	137	NH1	ARG	23	43.567	54.669	-6.006	1.00	29.51	A1
ATOM	138	HH11	ARG	23	43.562	55.377	-5.303	1.00	0.00	A1
ATOM	139	HH12	ARG	23	42.956	54.730	-6.789	1.00	0.00	A1
ATOM	140	NH2	ARG	23	44.345	52.604	-6.891	1.00	24.22	A1
ATOM	141	HH21	ARG	23	43.780	52.713	-7.709	1.00	0.00	A1
ATOM	142	HH22	ARG	23	44.936	51.802	-6.793	1.00	0.00	A1
ATOM	143	C	ARG	23	45.458	57.285	-0.560	1.00	20.56	A1
ATOM	144	O	ARG	23	44.374	57.254	0.042	1.00	20.04	A1
ATOM	145	N	LYS	24	46.485	58.015	-0.118	1.00	22.67	A1
ATOM	146	H	LYS	24	47.291	58.105	-0.668	1.00	0.00	A1
ATOM	147	CA	LYS	24	46.431	58.729	1.166	1.00	22.85	A1
ATOM	148	CB	LYS	24	47.811	59.255	1.506	1.00	26.86	A1
ATOM	149	CG	LYS	24	47.821	59.661	2.971	1.00	33.79	A1
ATOM	150	CD	LYS	24	49.121	60.265	3.404	1.00	40.73	A1
ATOM	151	CE	LYS	24	50.258	59.258	3.335	1.00	46.19	A1
ATOM	152	NZ	LYS	24	51.532	59.975	3.333	1.00	51.19	A1
ATOM	153	HZ1	LYS	24	51.637	60.498	4.225	1.00	0.00	A1
ATOM	154	HZ2	LYS	24	51.539	60.651	2.539	1.00	0.00	A1
ATOM	155	HZ3	LYS	24	52.317	59.303	3.216	1.00	0.00	A1
ATOM	156	C	LYS	24	45.455	59.893	1.101	1.00	21.66	A1
ATOM	157	O	LYS	24	44.588	60.068	1.962	1.00	20.90	A1
ATOM	158	N	ILE	25	45.549	60.696	0.044	1.00	21.66	A1
ATOM	159	H	ILE	25	46.242	60.509	-0.629	1.00	0.00	A1
ATOM	160	CA	ILE	25	44.667	61.841	-0.115	1.00	22.53	A1
ATOM	161	CB	ILE	25	45.075	62.694	-1.307	1.00	22.15	A1
ATOM	162	CG2	ILE	25	44.097	63.834	-1.439	1.00	20.44	A1
ATOM	163	CG1	ILE	25	46.475	63.230	-1.136	1.00	21.06	A1
ATOM	164	CD	ILE	25	47.188	63.281	-2.497	1.00	20.03	A1
ATOM	165	C	ILE	25	43.263	61.308	-0.352	1.00	21.75	A1
ATOM	166	O	ILE	25	42.339	61.839	0.301	1.00	26.14	A1
ATOM	167	N	GLN	26	43.065	60.289	-1.244	1.00	22.79	A1
ATOM	168	H	GLN	26	43.842	59.926	-1.726	1.00	0.00	A1
ATOM	169	CA	GLN	26	41.737	59.713	-1.437	1.00	20.12	A1
ATOM	170	CB	GLN	26	41.729	58.539	-2.341	1.00	18.89	A1
ATOM	171	CG	GLN	26	42.203	59.042	-3.627	1.00	19.77	A1
ATOM	172	CD	GLN	26	42.163	57.996	-4.684	1.00	24.26	A1
ATOM	173	OE1	GLN	26	42.550	56.853	-4.465	1.00	25.82	A1
ATOM	174	NE2	GLN	26	41.732	58.351	-5.890	1.00	27.68	A1
ATOM	175	HE21	GLN	26	41.421	59.265	-6.042	1.00	0.00	A1
ATOM	176	HE22	GLN	26	41.743	57.649	-6.552	1.00	0.00	A1
ATOM	177	C	GLN	26	41.207	59.239	-0.111	1.00	21.68	A1
ATOM	178	O	GLN	26	40.067	59.550	0.220	1.00	27.02	A1
ATOM	179	N	GLY	27	41.952	58.622	0.773	1.00	22.54	A1
ATOM	180	H	GLY	27	42.891	58.420	0.575	1.00	0.00	A1
ATOM	181	CA	GLY	27	41.386	58.191	2.037	1.00	25.55	A1
ATOM	182	C	GLY	27	40.936	59.352	2.890	1.00	27.80	A1
ATOM	183	O	GLY	27	39.889	59.251	3.526	1.00	29.95	A1
ATOM	184	N	ASP	28	41.683	60.460	2.915	1.00	29.39	A1
ATOM	185	H	ASP	28	42.547	60.454	2.448	1.00	0.00	A1
ATOM	186	CA	ASP	28	41.257	61.680	3.624	1.00	28.45	A1
ATOM	187	CB	ASP	28	42.266	62.789	3.552	1.00	30.11	A1
ATOM	188	CG	ASP	28	43.737	62.502	3.777	1.00	31.72	A1
ATOM	189	OD1	ASP	28	44.539	63.074	2.995	1.00	41.95	A1
ATOM	190	OD2	ASP	28	44.063	61.811	4.741	1.00	42.00	A1
ATOM	191	C	ASP	28	39.994	62.264	2.960	1.00	25.81	A1
ATOM	192	O	ASP	28	39.101	62.699	3.655	1.00	26.21	A1
ATOM	193	N	GLY	29	39.882	62.270	1.631	1.00	23.91	A1
ATOM	194	H	GLY	29	40.660	61.950	1.135	1.00	0.00	A1
ATOM	195	CA	GLY	29	38.729	62.694	0.886	1.00	25.69	A1
ATOM	196	C	GLY	29	37.528	61.961	1.418	1.00	22.16	A1
ATOM	197	O	GLY	29	36.648	62.558	2.061	1.00	28.14	A1
ATOM	198	N	ALA	30	37.646	60.628	1.295	1.00	27.85	A1
ATOM	199	H	ALA	30	38.442	60.288	0.843	1.00	0.00	A1
ATOM	200	CA	ALA	30	36.683	59.655	1.814	1.00	25.94	A1
ATOM	201	CB	ALA	30	37.269	58.303	1.556	1.00	22.15	A1
ATOM	202	C	ALA	30	36.356	59.842	3.308	1.00	27.18	A1

FIGURE 5

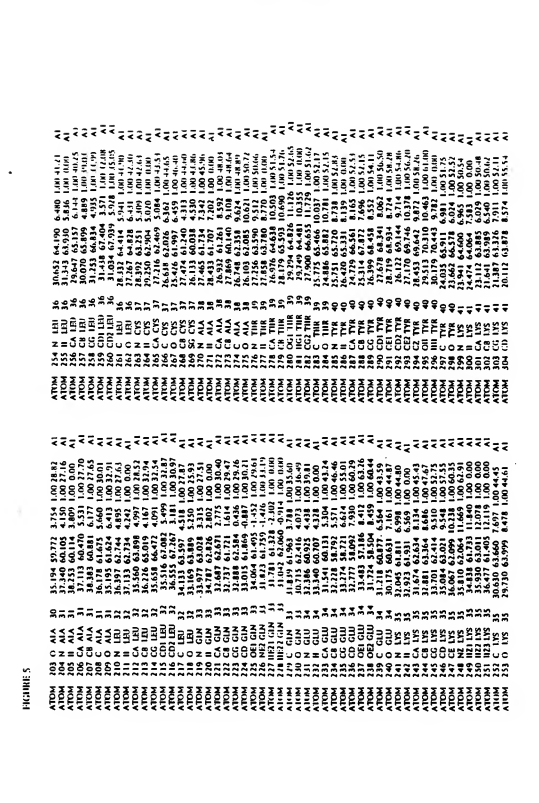

FIGURE 5

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ATOM  305 CE  LYS  41     19.578  63.087   9.820  1.00 58.79      A1
ATOM  306 NZ  LYS  41     18.374  63.648  10.457  1.00 58.31      A1
ATOM  307 1H1 LYS  41     17.605  63.688   9.757  1.00  0.00      A1
ATOM  308 1H2 LYS  41     18.578  64.607  10.803  1.00  0.00      A1
ATOM  309 1H3 LYS  41     18.084  63.043  11.252  1.00  0.00      A1
ATOM  310 C   LYS  41     23.251  64.318   4.586  1.00 49.92      A1
ATOM  311 O   LYS  41     22.312  64.124   3.793  1.00 51.49      A1
ATOM  312 N   LEU  42     24.432  64.893   4.246  1.00 48.28      A1
ATOM  313 H   LEU  42     25.103  65.050   4.937  1.00  0.00      A1
ATOM  314 CA  LEU  42     24.742  65.286   2.859  1.00 46.61      A1
ATOM  315 CB  LEU  42     25.565  66.574   2.757  1.00 44.69      A1
ATOM  316 CG  LEU  42     24.807  67.802   3.218  1.00 42.63      A1
ATOM  317 CD1 LEU  42     25.718  68.580   4.097  1.00 43.29      A1
ATOM  318 CD2 LEU  42     24.283  68.590   2.045  1.00 41.26      A1
ATOM  319 C   LEU  42     25.580  64.124   2.397  1.00 45.46      A1
ATOM  320 O   LEU  42     26.766  64.017   2.711  1.00 46.32      A1
ATOM  321 N   CYS  43     24.882  63.193   1.754  1.00 44.09      A1
ATOM  322 H   CYS  43     23.925  63.353   1.619  1.00  0.00      A1
ATOM  323 CA  CYS  43     25.480  61.951   1.358  1.00 42.87      A1
ATOM  324 C   CYS  43     25.448  61.846  -0.123  1.00 41.62      A1
ATOM  325 O   CYS  43     25.762  60.805  -0.666  1.00 41.99      A1
ATOM  326 CB  CYS  43     24.716  60.796   2.026  1.00 41.77      A1
ATOM  327 SG  CYS  43     24.523  61.011   3.835  1.00 45.91      A1
ATOM  328 N   HIS  44     25.057  62.846  -0.881  1.00 42.90      A1
ATOM  329 H   HIS  44     24.841  63.721  -0.491  1.00  0.00      A1
ATOM  330 CA  HIS  44     25.069  62.680  -2.320  1.00 44.60      A1
ATOM  331 CB  HIS  44     23.653  62.264  -2.825  1.00 48.40      A1
ATOM  332 CG  HIS  44     23.085  60.935  -2.310  1.00 50.37      A1
ATOM  333 CD2 HIS  44     22.178  60.844  -1.272  1.00 50.52      A1
ATOM  334 ND1 HIS  44     23.358  59.689  -2.713  1.00 52.28      A1
ATOM  335 HD1 HIS  44     24.130  59.396  -3.251  1.00  0.00      A1
ATOM  336 CE1 HIS  44     22.652  58.873  -1.955  1.00 51.92      A1
ATOM  337 NE2 HIS  44     21.947  59.565  -1.091  1.00 50.53      A1
ATOM  338 HE2 HIS  44     21.290  59.189  -0.466  1.00  0.00      A1
ATOM  339 C   HIS  44     25.522  63.941  -3.047  1.00 43.69      A1
ATOM  340 O   HIS  44     24.765  64.906  -3.108  1.00 43.00      A1
ATOM  341 N   PRO  45     26.710  63.978  -3.667  1.00 43.07      A1
ATOM  342 CD  PRO  45     27.785  62.995  -3.501  1.00 42.17      A1
ATOM  343 CA  PRO  45     27.133  65.074  -4.570  1.00 42.50      A1
ATOM  344 CB  PRO  45     28.380  64.466  -5.217  1.00 39.76      A1
ATOM  345 CG  PRO  45     28.995  63.680  -4.123  1.00 39.09      A1
ATOM  346 C   PRO  45     26.071  65.423  -5.585  1.00 44.49      A1
ATOM  347 O   PRO  45     25.876  66.612  -5.801  1.00 45.36      A1
ATOM  348 N   GLU  46     25.334  64.501  -6.225  1.00 45.36      A1
ATOM  349 H   GLU  46     25.464  63.561  -5.996  1.00  0.00      A1
ATOM  350 CA  GLU  46     24.406  64.806  -7.319  1.00 45.46      A1
ATOM  351 CB  GLU  46     23.952  63.515  -7.997  1.00 50.54      A1
ATOM  352 CG  GLU  46     24.462  63.460  -9.445  1.00 58.48      A1
ATOM  353 CD  GLU  46     23.637  64.215 -10.502  1.00 64.93      A1
ATOM  354 OE1 GLU  46     23.642  65.455 -10.516  1.00 68.55      A1
ATOM  355 OE2 GLU  46     22.995  63.554 -11.332  1.00 68.11      A1

ATOM  356 C   GLU  46     23.181  65.584  -6.937  1.00 42.96      A1
ATOM  357 O   GLU  46     22.532  66.223  -7.748  1.00 41.71      A1
ATOM  358 N   GLU  47     22.919  65.563  -5.654  1.00 41.96      A1
ATOM  359 H   GLU  47     23.507  65.098  -5.028  1.00  0.00      A1
ATOM  360 CA  GLU  47     21.818  66.301  -5.144  1.00 41.21      A1
ATOM  361 CB  GLU  47     21.294  65.487  -3.963  1.00 45.24      A1
ATOM  362 CG  GLU  47     21.409  65.925  -2.515  1.00 46.07      A1
ATOM  363 CD  GLU  47     20.812  64.907  -1.547  1.00 47.86      A1
ATOM  364 OE1 GLU  47     19.847  64.225  -1.910  1.00 50.99      A1
ATOM  365 OE2 GLU  47     21.313  64.780  -0.427  1.00 49.47      A1
ATOM  366 C   GLU  47     22.295  67.718  -4.809  1.00 43.04      A1
ATOM  367 O   GLU  47     21.532  68.547  -4.292  1.00 44.60      A1
ATOM  368 N   LEU  48     23.567  68.015  -5.121  1.00 43.05      A1
ATOM  369 H   LEU  48     24.140  67.310  -5.465  1.00  0.00      A1
ATOM  370 CA  LEU  48     24.166  69.318  -4.904  1.00 42.42      A1
ATOM  371 CB  LEU  48     25.223  69.201  -3.858  1.00 40.55      A1
ATOM  372 CG  LEU  48     24.920  68.695  -2.489  1.00 41.87      A1
ATOM  373 CD1 LEU  48     26.277  68.424  -1.892  1.00 41.71      A1
ATOM  374 CD2 LEU  48     24.096  69.670  -1.633  1.00 41.13      A1
ATOM  375 C   LEU  48     24.792  69.937  -6.166  1.00 42.37      A1
ATOM  376 O   LEU  48     25.439  70.994  -6.098  1.00 42.47      A1
ATOM  377 N   VAL  49     24.586  69.366  -7.347  1.00 41.52      A1
ATOM  378 H   VAL  49     23.951  68.602  -7.362  1.00  0.00      A1
ATOM  379 CA  VAL  49     25.191  69.822  -8.578  1.00 43.44      A1
ATOM  380 CB  VAL  49     24.590  68.761  -9.636  1.00 44.29      A1
ATOM  381 CG1 VAL  49     23.381  68.709  -9.830  1.00 47.50      A1
ATOM  382 CG2 VAL  49     25.540  69.086 -10.975  1.00 45.25      A1
ATOM  383 C   VAL  49     24.740  71.214  -9.028  1.00 44.98      A1
ATOM  384 O   VAL  49     25.401  71.901  -9.814  1.00 46.03      A1
ATOM  385 N   LEU  50     23.565  71.602  -8.530  1.00 46.16      A1
ATOM  386 H   LEU  50     23.081  70.933  -8.006  1.00  0.00      A1
ATOM  387 CA  LEU  50     22.908  72.895  -8.729  1.00 46.03      A1
ATOM  388 CB  LEU  50     21.469  72.769  -8.264  1.00 46.45      A1
ATOM  389 CG  LEU  50     20.443  73.718  -8.760  1.00 44.16      A1
ATOM  390 CD1 LEU  50     20.259  73.558 -10.243  1.00 44.79      A1
ATOM  391 CD2 LEU  50     19.159  73.400  -8.079  1.00 44.66      A1
ATOM  392 C   LEU  50     23.632  73.968  -7.917  1.00 45.85      A1
ATOM  393 O   LEU  50     23.996  74.989  -8.484  1.00 44.52      A1
ATOM  394 N   LEU  51     23.853  73.764  -6.606  1.00 45.44      A1
ATOM  395 H   LEU  51     23.489  72.958  -6.189  1.00  0.00      A1
ATOM  396 CA  LEU  51     24.676  74.656  -5.805  1.00 46.04      A1
ATOM  397 CB  LEU  51     24.860  74.084  -4.435  1.00 45.53      A1
ATOM  398 CG  LEU  51     25.741  74.931  -3.535  1.00 47.78      A1
ATOM  399 CD1 LEU  51     25.148  76.320  -3.322  1.00 47.13      A1
ATOM  400 CD2 LEU  51     25.902  74.202  -2.219  1.00 46.33      A1
ATOM  401 C   LEU  51     26.064  74.845  -6.416  1.00 46.27      A1
ATOM  402 O   LEU  51     26.551  75.966  -6.612  1.00 47.62      A1
ATOM  403 N   GLY  52     26.702  73.736  -6.809  1.00 44.84      A1
ATOM  404 H   GLY  52     26.306  72.869  -6.578  1.00  0.00      A1
ATOM  405 CA  GLY  52     27.989  73.758  -7.453  1.00 42.71      A1
ATOM  406 C   GLY  52     27.984  74.533  -8.750  1.00 42.47      A1
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EP 0 612 846 A1

FIGURE 5

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ATOM  407 O  GLY  52    28.853  75.364  -8.983  1.00 42.06      A1
ATOM  408 N  IHS  53    27.047  74.307  -9.653  1.00 42.02      A1
ATOM  409 H  IHS  53    26.366  73.624  -9.471  1.00  0.00      A1
ATOM  410 CA IHS  53    27.009  75.104 -10.861  1.00 42.23      A1
ATOM  411 CB IHS  53    25.842  74.689 -11.706  1.00 42.21      A1
ATOM  412 CG IHS  53    26.076  73.399 -12.460  1.00 44.60      A1
ATOM  413 CD2 IHS  53    25.112  72.774 -13.200  1.00 47.49      A1
ATOM  414 ND1 IHS  53    27.180  72.669 -12.578  1.00 46.76      A1
ATOM  415 HD1 IHS  53    28.039  72.853 -12.139  1.00  0.00      A1
ATOM  416 CE1 IHS  53    26.954  71.641 -13.346  1.00 46.90      A1
ATOM  417 NE2 IHS  53    25.704  71.725 -13.707  1.00 50.22      A1
ATOM  418 HE2 IHS  53    25.237  71.033 -14.239  1.00  0.00      A1
ATOM  419 C  IHS  53    26.893  76.585 -10.536  1.00 42.72      A1
ATOM  420 O  IHS  53    27.622  77.399 -11.068  1.00 42.03      A1
ATOM  421 N  SER  54    26.099  76.920  -9.535  1.00 45.08      A1
ATOM  422 H  SER  54    25.673  76.218  -9.001  1.00  0.00      A1
ATOM  423 CA SER  54    25.392  78.178  -9.177  1.00 46.92      A1
ATOM  424 CB SER  54    24.576  78.181  -8.289  1.00 48.86      A1
ATOM  425 OG SER  54    23.521  77.616  -9.112  1.00 53.06      A1
ATOM  426 HG SER  54    23.465  76.677  -8.918  1.00  0.00      A1
ATOM  427 C  SER  54    26.939  79.033  -8.549  1.00 47.92      A1
ATOM  428 O  SER  54    27.038  80.266  -8.655  1.00 49.60      A1
ATOM  429 N  LEU  55    27.837  78.273  -7.933  1.00 47.59      A1
ATOM  430 H  LEU  55    27.638  77.322  -7.791  1.00  0.00      A1
ATOM  431 CA LEU  55    29.075  78.810  -7.401  1.00 45.27      A1
ATOM  432 CB LEU  55    29.552  77.913  -6.243  1.00 45.49      A1
ATOM  433 CG LEU  55    28.840  77.992  -4.874  1.00 47.30      A1
ATOM  434 CD1 LEU  55    28.876  76.596  -4.199  1.00 49.53      A1
ATOM  435 CD2 LEU  55    29.530  78.921  -3.862  1.00 45.69      A1
ATOM  436 C  LEU  55    30.133  78.889  -8.492  1.00 43.63      A1
ATOM  437 O  LEU  55    31.247  79.350  -8.277  1.00 43.24      A1
ATOM  438 N  GLY  56    29.855  78.383  -9.675  1.00 43.55      A1
ATOM  439 H  GLY  56    28.984  77.975  -9.828  1.00  0.00      A1
ATOM  440 CA GLY  56    30.814  78.390 -10.753  1.00 45.20      A1
ATOM  441 C  GLY  56    32.187  77.811 -10.392  1.00 46.76      A1
ATOM  442 O  GLY  56    33.171  78.213 -11.015  1.00 47.31      A1
ATOM  443 N  ILE  57    32.247  76.885  -9.412  1.00 47.44      A1
ATOM  444 H  ILE  57    31.392  76.594  -9.047  1.00  0.00      A1
ATOM  445 CA ILE  57    33.486  76.249  -8.950  1.00 48.28      A1
ATOM  446 CB ILE  57    33.144  75.172  -7.863  1.00 47.79      A1
ATOM  447 CG2 ILE  57    34.457  74.591  -7.348  1.00 46.85      A1
ATOM  448 CG1 ILE  57    32.318  75.764  -6.701  1.00 45.09      A1
ATOM  449 CD ILE  57    31.859  74.739  -5.659  1.00 41.23      A1
ATOM  450 C  ILE  57    34.276  75.602 -10.115  1.00 49.15      A1
ATOM  451 O  ILE  57    33.678  74.935 -10.968  1.00 49.04      A1
ATOM  452 N  PRO  58    35.596  75.817 -10.248  1.00 49.75      A1
ATOM  453 CD PRO  58    36.402  76.743  -9.433  1.00 50.94      A1
ATOM  454 CA PRO  58    36.421  75.228 -11.302  1.00 50.12      A1
ATOM  455 CB PRO  58    37.525  76.241 -11.488  1.00 50.91      A1
ATOM  456 CG PRO  58    37.814  76.663 -10.041  1.00 50.82      A1
ATOM  457 C  PRO  58    36.916  73.845 -11.875  1.00 50.36      A1
ATOM  458 O  PRO  58    37.187  73.599  -9.691  1.00 49.75      A1
ATOM  459 N  TRP  59    37.030  72.927 -11.816  1.00 50.37      A1
ATOM  460 H  TRP  59    36.888  73.141 -12.760  1.00  0.00      A1
ATOM  461 CA TRP  59    37.524  71.595 -11.482  1.00 51.78      A1
ATOM  462 CB TRP  59    36.435  70.562 -11.857  1.00 49.06      A1
ATOM  463 CG TRP  59    35.254  70.712 -10.889  1.00 46.17      A1
ATOM  464 CD2 TRP  59    35.320  70.845  -9.521  1.00 44.06      A1
ATOM  465 CE2 TRP  59    33.998  71.027  -9.105  1.00 44.18      A1
ATOM  466 CE3 TRP  59    36.274  70.842  -8.538  1.00 44.01      A1
ATOM  467 CD1 TRP  59    33.972  70.794 -11.354  1.00 45.17      A1
ATOM  468 NE1 TRP  59    33.229  70.994 -10.197  1.00 43.17      A1
ATOM  469 HE1 TRP  59    32.301  71.312 -10.332  1.00  0.00      A1
ATOM  470 CZ2 TRP  59    33.598  71.215  -7.916  1.00 45.60      A1
ATOM  471 CZ3 TRP  59    35.893  71.028  -7.243  1.00 45.25      A1
ATOM  472 CH2 TRP  59    34.565  71.214  -6.938  1.00 46.44      A1
ATOM  473 C  TRP  59    38.815  71.435 -12.256  1.00 52.84      A1
ATOM  474 O  TRP  59    38.842  71.972 -13.372  1.00 54.96      A1
ATOM  475 N  ALA  60    39.912  70.834 -11.777  1.00 51.97      A1
ATOM  476 H  ALA  60    39.857  70.269 -10.977  1.00  0.00      A1
ATOM  477 CA ALA  60    41.108  70.870 -12.609  1.00 52.18      A1
ATOM  478 CB ALA  60    42.303  70.610 -11.748  1.00 51.75      A1
ATOM  479 C  ALA  60    41.055  69.857 -13.746  1.00 52.16      A1
ATOM  480 O  ALA  60    40.545  68.760 -13.530  1.00 52.17      A1
ATOM  481 N  PRO  61    41.435  70.145 -14.986  1.00 53.14      A1
ATOM  482 CD PRO  61    41.370  71.458 -15.622  1.00 54.76      A1
ATOM  483 CA PRO  61    41.691  69.145 -15.993  1.00 55.57      A1
ATOM  484 CB PRO  61    41.792  69.918 -17.310  1.00 54.95      A1
ATOM  485 CG PRO  61    41.311  71.297 -16.901  1.00 54.05      A1
ATOM  486 C  PRO  61    42.934  68.333 -15.690  1.00 57.54      A1
ATOM  487 O  PRO  61    43.757  68.661 -14.834  1.00 57.20      A1
ATOM  488 N  LEU  62    43.040  67.271 -16.486  1.00 59.98      A1
ATOM  489 H  LEU  62    42.285  67.067 -17.077  1.00  0.00      A1
ATOM  490 CA LEU  62    44.184  66.370 -16.471  1.00 63.64      A1
ATOM  491 CB LEU  62    44.062  65.417 -15.260  1.00 63.72      A1
ATOM  492 CG LEU  62    45.333  64.691 -14.865  1.00 64.43      A1
ATOM  493 CD1 LEU  62    46.394  65.704 -14.488  1.00 64.02      A1
ATOM  494 CD2 LEU  62    45.016  63.764 -13.717  1.00 64.98      A1
ATOM  495 C  LEU  62    44.214  65.611 -17.812  1.00 65.09      A1
ATOM  496 OT1 LEU  62    44.256  66.302 -18.844  1.00 68.47      A1
ATOM  497 OT2 LEU  62    44.194  64.371 -17.845  1.00 66.57      A1
ATOM  498 CB LEU  72    37.448  63.159 -19.422  1.00 63.44      A/
ATOM  499 CG LEU  72    37.716  63.495 -18.117  1.00 63.40      A/
ATOM  500 CD1 LEU  72    36.719  61.408 -17.913  1.00 63.50      A/
ATOM  501 CD2 LEU  72    39.107  61.901 -18.121  1.00 63.22      A/
ATOM  502 C  LEU  72    35.897  65.084 -18.876  1.00 65.40      A/
ATOM  503 O  LEU  72    34.827  65.301 -18.316  1.00 67.30      A/
ATOM  504 HT1 LEU  72    36.469  64.083 -21.261  1.00  0.00      A/
ATOM  505 HT2 LEU  72    34.827  64.355 -20.951  1.00  0.00      A/
ATOM  506 N  LEU  72    35.795  63.983 -20.899  1.00 66.29      A/
ATOM  507 HT3 LEU  72    35.866  63.198 -21.449  1.00  0.00      A/
ATOM  508 CA LEU  72    36.064  63.714 -19.512  1.00 64.91      A/
```

FIGURE 5

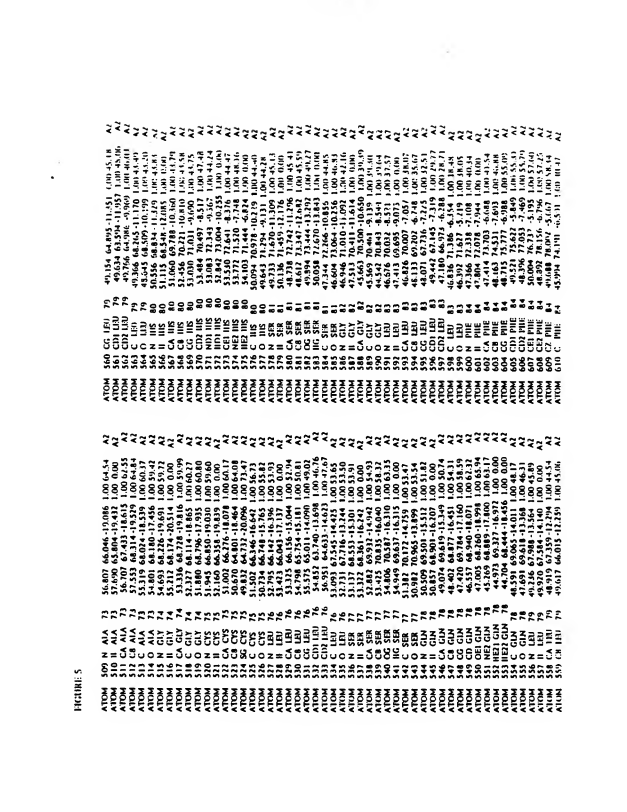

FIGURE 5

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ATOM   611 O  PHE  84     45.609 74.749 -5.558  1.00 42.71      A2
ATOM   612 N  LEU  85     45.190 73.953 -7.624  1.00 38.64      A2
ATOM   613 H  LEU  85     45.555 73.537 -8.479  1.00  0.00      A2
ATOM   614 CA LEU  85     43.794 74.335 -7.584  1.00 39.81      A2
ATOM   615 CB LEU  85     43.101 73.886 -8.839  1.00 41.27      A2
ATOM   616 CG LEU  85     41.673 74.403 -9.017  1.00 46.45      A2
ATOM   617 CD1 LEU  85     41.702 75.784 -9.719  1.00 47.80      A2
ATOM   618 CD2 LEU  85     40.860 73.359 -9.787  1.00 48.25      A2
ATOM   619 C  LEU  85     43.079 73.731 -6.386  1.00 38.20      A2
ATOM   620 O  LEU  85     42.498 74.460 -5.582  1.00 38.36      A2
ATOM   621 N  TYR  86     43.150 72.405 -6.198  1.00 37.92      A2
ATOM   622 H  TYR  86     43.637 71.850 -6.845  1.00  0.00      A2
ATOM   623 CA TYR  86     42.501 71.801 -5.057  1.00 37.15      A2
ATOM   624 CB TYR  86     42.598 70.255 -5.102  1.00 36.73      A2
ATOM   625 CG TYR  86     41.561 69.685 -6.081  1.00 33.66      A2
ATOM   626 CD1 TYR  86     41.946 69.312 -7.374  1.00 33.03      A2
ATOM   627 CE1 TYR  86     40.991 68.885 -8.280  1.00 30.08      A2
ATOM   628 CD2 TYR  86     40.224 69.623 -5.666  1.00 32.61      A2
ATOM   629 CE2 TYR  86     39.263 69.203 -6.574  1.00 31.66      A2
ATOM   630 CZ TYR  86     39.656 68.838 -7.868  1.00 30.57      A2
ATOM   631 OH TYR  86     38.670 68.428 -8.751  1.00 28.18      A2
ATOM   632 HH TYR  86     39.107 67.994 -9.485  1.00  0.00      A2
ATOM   633 C  TYR  86     43.054 72.318 -3.746  1.00 37.75      A2
ATOM   634 O  TYR  86     42.173 72.469 -2.889  1.00 39.52      A2
ATOM   635 N  GLN  87     44.347 72.655 -3.478  1.00 36.93      A2
ATOM   636 H  GLN  87     45.044 72.463 -4.140  1.00  0.00      A2
ATOM   637 CA GLN  87     44.749 73.332 -2.205  1.00 36.40      A2
ATOM   638 CB GLN  87     46.210 73.668 -2.255  1.00 39.56      A2
ATOM   639 CG GLN  87     47.126 72.993 -1.237  1.00 46.99      A2
ATOM   640 CD GLN  87     48.641 73.062 -1.576  1.00 50.96      A2
ATOM   641 OE1 GLN  87     49.144 72.623 -2.627  1.00 52.15      A2
ATOM   642 NE2 GLN  87     49.446 73.608 -0.663  1.00 52.96      A2
ATOM   643 HE2 GLN  87     49.055 73.957 0.164  1.00  0.00      A2
ATOM   644 HE22 GLN  87     50.396 73.621 -0.888  1.00  0.00      A2
ATOM   645 C  GLN  87     43.941 74.652 -2.013  1.00 34.36      A2
ATOM   646 O  GLN  87     43.414 74.990 -0.935  1.00 31.55      A2
ATOM   647 N  GLY  88     43.740 75.335 -3.159  1.00 32.73      A2
ATOM   648 H  GLY  88     44.165 75.005 -3.981  1.00  0.00      A2
ATOM   649 CA GLY  88     42.948 76.546 -3.232  1.00 30.81      A2
ATOM   650 C  GLY  88     41.540 76.275 -2.731  1.00 30.47      A2
ATOM   651 O  GLY  88     41.130 76.819 -1.703  1.00 30.27      A2
ATOM   652 N  LEU  89     40.802 75.387 -3.406  1.00 29.01      A2
ATOM   653 H  LEU  89     41.220 74.912 -4.154  1.00  0.00      A2
ATOM   654 CA LEU  89     39.447 75.102 -3.009  1.00 27.60      A2
ATOM   655 CB LEU  89     38.922 74.073 -3.935  1.00 28.13      A2
ATOM   656 CG LEU  89     38.764 74.583 -5.340  1.00 29.51      A2
ATOM   657 CD1 LEU  89     38.363 73.530 -6.364  1.00 24.13      A2
ATOM   658 CD2 LEU  89     37.673 75.637 -5.220  1.00 32.87      A2
ATOM   659 C  LEU  89     39.352 74.629 -1.583  1.00 29.88      A2
ATOM   660 O  LEU  89     38.427 75.012 -0.860  1.00 30.81      A2
ATOM   661 N  LEU  90     40.317 73.839 -1.094  1.00 31.59      A2
ATOM   662 H  LEU  90     41.101 73.626 -1.643  1.00  0.00      A2
ATOM   663 CA LEU  90     40.182 73.274  0.235  1.00 33.41      A2
ATOM   664 CB LEU  90     41.207 72.234  0.501  1.00 36.15      A2
ATOM   665 CG LEU  90     41.075 70.971 -0.343  1.00 38.76      A2
ATOM   666 CD1 LEU  90     42.431 70.267 -0.456  1.00 37.21      A2
ATOM   667 CD2 LEU  90     39.995 70.099  1.179  1.00 40.54      A2
ATOM   668 C  LEU  90     40.342 74.319  1.255  1.00 34.21      A2
ATOM   669 O  LEU  90     39.711 74.256  2.313  1.00 35.57      A2
ATOM   670 N  GLN  91     41.188 75.291  0.940  1.00 35.24      A2
ATOM   671 H  GLN  91     41.663 75.284  0.078  1.00  0.00      A2
ATOM   672 CA GLN  91     41.397 76.373  1.883  1.00 37.40      A2
ATOM   673 CB GLN  91     42.557 77.182  1.363  1.00 39.65      A2
ATOM   674 CG GLN  91     43.155 78.237  2.284  1.00 44.37      A2
ATOM   675 CD GLN  91     44.348 78.799  1.542  1.00 46.96      A2
ATOM   676 OE1 GLN  91     45.235 78.083  1.068  1.00 47.42      A2
ATOM   677 NE2 GLN  91     44.376 80.092  1.341  1.00 46.87      A2
ATOM   678 HE21 GLN  91     43.690 80.685  1.700  1.00  0.00      A2
ATOM   679 HE22 GLN  91     45.108 80.331  0.741  1.00  0.00      A2
ATOM   680 C  GLN  91     40.129 77.231  2.061  1.00 37.32      A2
ATOM   681 O  GLN  91     39.718 77.530  3.186  1.00 36.21      A2
ATOM   682 N  ALA  92     39.456 77.570  0.943  1.00 38.65      A2
ATOM   683 H  ALA  92     39.808 77.205  0.098  1.00  0.00      A2
ATOM   684 CA ALA  92     38.243 78.402  0.880  1.00 39.10      A2
ATOM   685 CB ALA  92     37.657 78.436 -0.511  1.00 36.76      A2
ATOM   686 C  ALA  92     37.139 77.905  1.770  1.00 38.95      A2
ATOM   687 O  ALA  92     36.794 78.687  2.194  1.00 42.45      A2
ATOM   688 N  LEU  93     37.151 76.618  2.123  1.00 38.14      A2
ATOM   689 H  LEU  93     37.855 76.040  1.759  1.00  0.00      A2
ATOM   690 CA LEU  93     36.111 76.018  2.972  1.00 36.90      A2
ATOM   691 CB LEU  93     36.008 74.463  2.794  1.00 35.24      A2
ATOM   692 CG LEU  93     35.725 73.992  1.378  1.00 33.55      A2
ATOM   693 CD1 LEU  93     36.159 72.583  1.129  1.00 33.26      A2
ATOM   694 CD2 LEU  93     34.254 74.167  1.115  1.00 32.16      A2
ATOM   695 C  LEU  93     36.764 76.353  4.426  1.00 36.44      A2
ATOM   696 O  LEU  93     35.473 75.917  5.256  1.00 35.17      A2
ATOM   697 N  GLU  94     37.357 77.019  4.736  1.00 38.19      A2
ATOM   698 H  GLU  94     38.022 77.167  4.035  1.00  0.00      A2
ATOM   699 CA GLU  94     37.627 77.573  6.038  1.00 42.71      A2
ATOM   700 CB GLU  94     36.931 78.947  6.165  1.00 47.18      A2
ATOM   701 CG GLU  94     37.418 80.011  5.131  1.00 56.10      A2
ATOM   702 CD GLU  94     36.423 81.153  4.862  1.00 60.26      A2
ATOM   703 OE1 GLU  94     35.728 81.109  3.823  1.00 60.76      A2
ATOM   704 OE2 GLU  94     36.331 82.054  5.721  1.00 61.64      A2
ATOM   705 C  GLU  94     37.245 76.701  7.198  1.00 43.90      A2
ATOM   706 O  GLU  94     36.624 77.172  8.167  1.00 45.70      A2
ATOM   707 N  GLY  95     37.641 75.410  7.001  1.00 44.05      A2
ATOM   708 H  GLY  95     38.024 75.192  6.127  1.00  0.00      A2
ATOM   709 CA GLY  95     37.319 74.310  7.981  1.00 42.49      A2
ATOM   710 C  GLY  95     36.162 73.612  8.061  1.00 42.24      A2
ATOM   711 O  GLY  95     36.038 72.596  8.739  1.00 40.02      A2
ATOM   712 N  ILE  96     35.160 74.123  7.328  1.00 42.82      A2
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FIGURE 5

ATOM	713	H	ILE	96	35.357	74.944	6.841	1.00	0.00	A2
ATOM	714	CA	ILE	96	33.760	73.692	7.312	1.00	42.12	A2
ATOM	715	CB	ILE	96	33.665	72.233	6.800	1.00	36.33	A2
ATOM	716	CG2	ILE	96	32.248	71.768	6.789	1.00	34.79	A2
ATOM	717	CG1	ILE	96	34.091	72.157	5.374	1.00	35.35	A2
ATOM	718	CD	ILE	96	34.051	70.743	4.738	1.00	33.64	A2
ATOM	719	C	ILE	96	33.106	73.863	8.709	1.00	44.74	A2
ATOM	720	O	ILE	96	32.220	74.716	8.841	1.00	44.59	A2
ATOM	721	N	SER	97	33.467	73.154	9.780	1.00	46.84	A2
ATOM	722	H	SER	97	34.243	72.553	9.706	1.00	0.00	A2
ATOM	723	CA	SER	97	32.900	73.359	11.105	1.00	48.91	A2
ATOM	724	CB	SER	97	31.804	72.343	11.347	1.00	49.60	A2
ATOM	725	OG	SER	97	32.211	71.120	11.954	1.00	52.85	A2
ATOM	726	HG	SER	97	31.406	70.573	11.942	1.00	0.00	A2
ATOM	727	C	SER	97	34.045	73.143	12.077	1.00	50.64	A2
ATOM	728	O	SER	97	35.035	72.538	11.678	1.00	52.78	A2
ATOM	729	N	PRO	98	34.063	73.474	13.348	1.00	52.12	A2
ATOM	730	CD	PRO	98	33.002	74.170	14.016	1.00	52.90	A2
ATOM	731	CA	PRO	98	35.195	73.200	14.257	1.00	54.94	A2
ATOM	732	CB	PRO	98	34.750	73.717	15.600	1.00	54.78	A2
ATOM	733	CG	PRO	98	33.772	74.777	15.182	1.00	55.48	A2
ATOM	734	C	PRO	98	35.591	71.723	14.336	1.00	56.75	A2
ATOM	735	O	PRO	98	36.738	71.274	14.468	1.00	57.85	A2
ATOM	736	N	GLU	99	34.509	70.971	14.214	1.00	58.21	A2
ATOM	737	H	GLU	99	33.652	71.400	14.028	1.00	0.00	A2
ATOM	738	CA	GLU	99	34.543	69.537	14.281	1.00	58.48	A2
ATOM	739	CB	GLU	99	33.111	69.104	14.304	1.00	63.30	A2
ATOM	740	CG	GLU	99	32.958	67.702	14.852	1.00	71.04	A2
ATOM	741	CD	GLU	99	32.076	66.838	13.962	1.00	76.95	A2
ATOM	742	OE1	GLU	99	32.209	65.608	14.079	1.00	80.63	A2
ATOM	743	OE2	GLU	99	31.295	67.382	13.153	1.00	77.99	A2
ATOM	744	C	GLU	99	35.298	69.025	13.074	1.00	55.31	A2
ATOM	745	O	GLU	99	36.251	68.270	13.210	1.00	55.96	A2
ATOM	746	N	LEU	100	34.916	69.475	11.891	1.00	51.23	A2
ATOM	747	H	LEU	100	34.214	70.159	11.841	1.00	0.00	A2
ATOM	748	CA	LEU	100	35.577	69.052	10.678	1.00	48.08	A2
ATOM	749	CB	LEU	100	34.627	69.341	9.574	1.00	45.52	A2
ATOM	750	CG	LEU	100	33.544	68.337	9.674	1.00	45.39	A2
ATOM	751	CD1	LEU	100	32.207	68.972	9.458	1.00	46.40	A2
ATOM	752	CD2	LEU	100	33.851	67.245	8.677	1.00	47.48	A2
ATOM	753	C	LEU	100	36.956	69.629	10.368	1.00	46.77	A2
ATOM	754	O	LEU	100	37.578	69.244	9.357	1.00	46.62	A2
ATOM	755	N	GLY	101	37.441	70.505	11.272	1.00	45.40	A2
ATOM	756	H	GLY	101	36.893	70.704	12.056	1.00	0.00	A2
ATOM	757	CA	GLY	101	38.703	71.238	11.126	1.00	42.52	A2
ATOM	758	C	GLY	101	39.885	70.334	10.798	1.00	40.73	A2
ATOM	759	O	GLY	101	40.475	70.402	9.710	1.00	40.69	A2
ATOM	760	N	PRO	102	40.250	69.441	11.708	1.00	38.61	A2
ATOM	761	CD	PRO	102	39.676	69.350	13.027	1.00	39.26	A2
ATOM	762	CA	PRO	102	41.390	68.566	11.606	1.00	37.30	A2
ATOM	763	CB	PRO	102	41.294	67.690	12.775	1.00	39.36	A2
ATOM	764	CG	PRO	102	40.799	68.687	13.776	1.00	41.02	A2
ATOM	765	C	PRO	102	41.364	67.795	10.331	1.00	37.15	A2
ATOM	766	O	PRO	102	42.358	67.854	9.600	1.00	38.88	A2
ATOM	767	N	THR	103	40.223	67.167	10.045	1.00	35.36	A2
ATOM	768	H	THR	103	39.466	67.223	10.662	1.00	0.00	A2
ATOM	769	CA	THR	103	40.051	66.386	8.843	1.00	34.62	A2
ATOM	770	CB	THR	103	38.592	65.888	8.715	1.00	34.07	A2
ATOM	771	OG1	THR	103	38.356	65.240	9.936	1.00	35.43	A2
ATOM	772	HG1	THR	103	38.011	65.896	10.548	1.00	0.00	A2
ATOM	773	CG2	THR	103	38.312	64.896	7.594	1.00	31.79	A2
ATOM	774	C	THR	103	40.417	67.215	7.625	1.00	34.61	A2
ATOM	775	O	THR	103	41.091	66.665	6.738	1.00	38.16	A2
ATOM	776	N	LEU	104	40.054	68.498	7.529	1.00	32.49	A2
ATOM	777	H	LEU	104	39.504	68.923	8.229	1.00	0.00	A2
ATOM	778	CA	LEU	104	40.471	69.267	6.370	1.00	30.49	A2
ATOM	779	CB	LEU	104	39.616	70.430	6.242	1.00	33.51	A2
ATOM	780	CG	LEU	104	38.356	69.996	5.611	1.00	36.61	A2
ATOM	781	CD1	LEU	104	37.222	70.621	6.381	1.00	39.43	A2
ATOM	782	CD2	LEU	104	38.418	70.294	4.132	1.00	37.69	A2
ATOM	783	C	LEU	104	41.904	69.727	6.414	1.00	28.48	A2
ATOM	784	O	LEU	104	42.583	69.825	5.398	1.00	28.47	A2
ATOM	785	N	ASP	105	42.449	69.949	7.574	1.00	26.99	A2
ATOM	786	H	ASP	105	41.903	69.912	8.388	1.00	0.00	A2
ATOM	787	CA	ASP	105	43.822	70.307	7.613	1.00	28.67	A2
ATOM	788	CB	ASP	105	44.139	70.584	9.038	1.00	33.06	A2
ATOM	789	CG	ASP	105	43.438	71.808	9.593	1.00	35.46	A2
ATOM	790	OD1	ASP	105	43.085	72.726	8.836	1.00	38.42	A2
ATOM	791	OD2	ASP	105	43.244	71.816	10.808	1.00	39.10	A2
ATOM	792	C	ASP	105	44.701	69.206	7.032	1.00	28.90	A2
ATOM	793	O	ASP	105	45.551	69.479	6.175	1.00	29.62	A2
ATOM	794	N	THR	106	44.415	67.950	7.401	1.00	26.86	A2
ATOM	795	H	THR	106	43.674	67.826	8.029	1.00	0.00	A2
ATOM	796	CA	THR	106	45.143	66.770	6.935	1.00	24.81	A2
ATOM	797	CB	THR	106	44.558	65.456	7.477	1.00	26.03	A2
ATOM	798	OG1	THR	106	44.680	65.566	8.894	1.00	31.53	A2
ATOM	799	HG1	THR	106	44.069	66.223	9.242	1.00	0.00	A2
ATOM	800	CG2	THR	106	45.258	64.220	7.011	1.00	20.90	A2
ATOM	801	C	THR	106	45.073	66.684	5.460	1.00	23.75	A2
ATOM	802	O	THR	106	46.065	66.411	4.812	1.00	24.68	A2
ATOM	803	N	LEU	107	43.887	66.917	4.946	1.00	24.30	A2
ATOM	804	H	LEU	107	43.145	67.176	5.528	1.00	0.00	A2
ATOM	805	CA	LEU	107	43.668	66.783	3.531	1.00	27.29	A2
ATOM	806	CB	LEU	107	42.158	66.913	3.273	1.00	25.45	A2
ATOM	807	CG	LEU	107	41.642	66.888	1.863	1.00	26.24	A2
ATOM	808	CD1	LEU	107	42.095	65.649	1.158	1.00	26.41	A2
ATOM	809	CD2	LEU	107	40.140	66.925	1.914	1.00	27.62	A2
ATOM	810	C	LEU	107	44.485	67.848	2.819	1.00	28.01	A2
ATOM	811	O	LEU	107	45.154	67.555	1.823	1.00	30.72	A2
ATOM	812	N	GLN	108	44.540	69.055	3.373	1.00	28.52	A2
ATOM	813	H	GLN	108	44.030	69.221	4.194	1.00	0.00	A2
ATOM	814	CA	GLN	108	45.343	70.132	2.792	1.00	28.08	A2

FIGURE 5

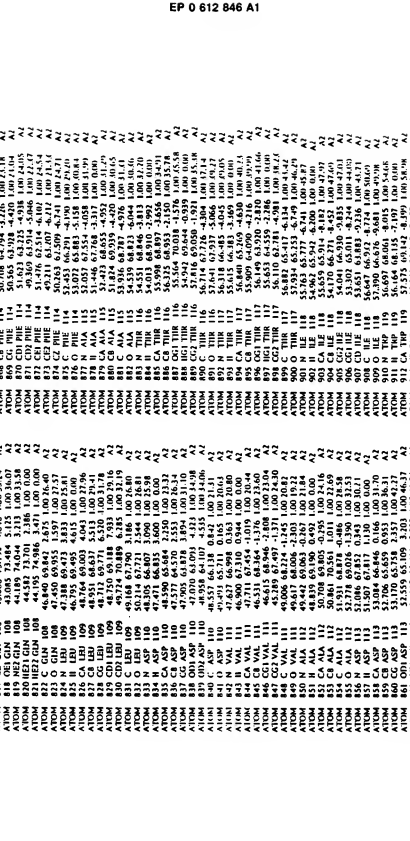

FIGURE 5

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ATOM  917 CE3 TRP 119      56.465 72.314 -10.080 1.00 66.02      A2
ATOM  918 CD1 TRP 119      59.322 71.870  -7.863 1.00 64.12      A2
ATOM  919 NE1 TRP 119      59.680 72.727  -8.784 1.00 65.00      A2
ATOM  920 HE1 TRP 119      60.568 73.140  -8.828 1.00  0.00      A2
ATOM  921 CZ2 TRP 119      58.726 73.794 -10.714 1.00 62.90      A2
ATOM  922 CZ3 TRP 119      56.469 73.157 -11.170 1.00 65.18      A2
ATOM  923 CH2 TRP 119      57.591 73.887 -11.481 1.00 64.40      A2
ATOM  924 C   TRP 119      59.021 68.664  -8.352 1.00 61.26      A2
ATOM  925 O   TRP 119      59.748 68.788  -9.343 1.00 62.12      A2
ATOM  926 N   GLN 120      59.447 68.065  -7.249 1.00 62.91      A2
ATOM  927 H   GLN 120      58.811 67.961  -6.519 1.00  0.00      A2
ATOM  928 CA  GLN 120      60.786 67.504  -7.113 1.00 65.06      A2
ATOM  929 CB  GLN 120      60.900 66.800  -5.780 1.00 66.56      A2
ATOM  930 CG  GLN 120      60.627 67.678  -4.582 1.00 67.18      A2
ATOM  931 CD  GLN 120      60.725 66.907  -3.284 1.00 67.17      A2
ATOM  932 OE1 GLN 120      61.221 67.465  -2.319 1.00 69.31      A2
ATOM  933 NE2 GLN 120      60.305 65.654  -3.129 1.00 67.39      A2
ATOM  934 HE21 GLN 120     59.903 65.174  -3.877 1.00  0.00      A2
ATOM  935 HE22 GLN 120     60.441 65.282  -2.234 1.00  0.00      A2
ATOM  936 C   GLN 120      61.169 66.509  -8.227 1.00 66.22      A2
ATOM  937 O   GLN 120      61.326 66.921  -9.662 1.00 66.50      A2
ATOM  938 N   GLN 121      60.202 65.745  -8.706 1.00 67.10      A2
ATOM  939 H   GLN 121      59.307 65.756  -8.303 1.00  0.00      A2
ATOM  940 CA  GLN 121      60.480 64.878  -9.812 1.00 66.66      A2
ATOM  941 CB  GLN 121      59.292 63.971 -10.070 1.00 67.96      A2
ATOM  942 CG  GLN 121      59.614 62.937 -11.128 1.00 68.89      A2
ATOM  943 CD  GLN 121      60.940 62.236 -10.852 1.00 71.37      A2
ATOM  944 OE1 GLN 121      61.212 61.706  -9.777 1.00 71.70      A2
ATOM  945 NE2 GLN 121      61.879 62.262 -11.786 1.00 74.41      A2
ATOM  946 HE21 GLN 121     61.703 62.729 -12.627 1.00 00.00      A2
ATOM  947 HE22 GLN 121     62.736 61.859 -11.541 1.00  0.00      A2
ATOM  948 C   GLN 121      60.760 65.743 -13.045 1.00 70.48      A2
ATOM  949 O   GLN 121      61.671 65.436 -11.827 1.00 70.94      A2
ATOM  950 N   MET 122      60.019 66.846 -11.236 1.00 71.67      A2
ATOM  951 H   MET 122      59.351 67.087 -10.555 1.00  0.00      A2
ATOM  952 CA  MET 122      60.190 67.688 -12.412 1.00 72.62      A2
ATOM  953 CB  MET 122      59.173 68.810 -12.448 1.00 73.12      A2
ATOM  954 CG  MET 122      57.880 68.343 -13.083 1.00 73.64      A2
ATOM  955 SD  MET 122      56.669 69.662 -13.295 1.00 75.44      A2
ATOM  956 CE  MET 122      55.695 69.349 -11.861 1.00 76.43      A2
ATOM  957 C   MET 122      61.566 68.281 -12.411 1.00 73.22      A2
ATOM  958 O   MET 122      62.240 68.287 -13.441 1.00 73.03      A2
ATOM  959 N   GLU 123      61.991 68.697 -11.223 1.00 74.74      A2
ATOM  960 H   GLU 123      61.372 68.617 -10.466 1.00  0.00      A2
ATOM  961 CA  GLU 123      63.305 69.262 -11.018 1.00 75.95      A2
ATOM  962 CB  GLU 123      63.484 69.665  -9.597 1.00 75.72      A2
ATOM  963 CG  GLU 123      62.644 70.906  -9.500 1.00 79.11      A2
ATOM  964 CD  GLU 123      62.651 71.529  -8.122 1.00 83.02      A2
ATOM  965 OE1 GLU 123      62.741 72.763  -8.057 1.00 84.15      A2
ATOM  966 OE2 GLU 123      62.543 70.789  -7.133 1.00 84.45      A2
ATOM  967 C   GLU 123      64.381 68.280 -11.386 1.00 77.17      A2
ATOM  968 O   GLU 123      65.092 68.558 -12.356 1.00 78.22      A2
ATOM  969 N   GLU 124      64.504 67.110 -10.765 1.00 77.66      A2
ATOM  970 H   GLU 124      63.867 66.852 -10.060 1.00  0.00      A2
ATOM  971 CA  GLU 124      65.574 66.215 -11.167 1.00 78.47      A2
ATOM  972 CB  GLU 124      65.600 65.051 -10.195 1.00 80.29      A2
ATOM  973 CG  GLU 124      64.387 64.132 -10.150 1.00 84.49      A2
ATOM  974 CD  GLU 124      64.375 63.248  -8.908 1.00 85.51      A2
ATOM  975 OE1 GLU 124      64.733 63.729  -7.824 1.00 86.84      A2
ATOM  976 OE2 GLU 124      64.006 62.075  -9.024 1.00 86.39      A2
ATOM  977 C   GLU 124      65.534 65.705 -12.612 1.00 78.01      A2
ATOM  978 O   GLU 124      66.480 65.057 -13.066 1.00 78.91      A2
ATOM  979 N   LEU 125      64.460 65.943 -13.363 1.00 77.11      A2
ATOM  980 H   LEU 125      63.666 66.340 -12.945 1.00  0.00      A2
ATOM  981 CA  LEU 125      64.387 65.583 -14.771 1.00 76.23      A2
ATOM  982 CB  LEU 125      63.061 64.832 -14.952 1.00 76.88      A2
ATOM  983 CG  LEU 125      62.392 64.382 -16.263 1.00 76.63      A2
ATOM  984 CD1 LEU 125      63.350 63.754 -17.276 1.00 76.67      A2
ATOM  985 CD2 LEU 125      61.309 63.402 -15.839 1.00 75.89      A2
ATOM  986 C   LEU 125      64.506 66.827 -15.648 1.00 75.84      A2
ATOM  987 O   LEU 125      64.360 66.788 -16.871 1.00 75.36      A2
ATOM  988 N   GLY 126      64.759 67.968 -15.027 1.00 75.90      A2
ATOM  989 H   GLY 126      64.741 67.976 -14.056 1.00  0.00      A2
ATOM  990 CA  GLY 126      64.968 69.213 -15.736 1.00 77.58      A2
ATOM  991 C   GLY 126      63.814 70.814 -16.330 1.00 78.63      A2
ATOM  992 O   GLY 126      63.735 70.736 -17.146 1.00 78.55      A2
ATOM  993 N   MET 127      62.524 69.343 -15.593 1.00 80.08      A2
ATOM  994 H   MET 127      62.522 68.603 -15.293 1.00  0.00      A2
ATOM  995 CA  MET 127      61.266 69.902 -16.415 1.00 81.46      A2
ATOM  996 CB  MET 127      60.191 68.802 -16.361 1.00 81.86      A2
ATOM  997 CG  MET 127      60.708 67.399 -17.147 1.00 81.66      A2
ATOM  998 SD  MET 127      59.682 66.115 -17.282 1.00 81.70      A2
ATOM  999 CE  MET 127      60.236 65.620 -18.900 1.00 83.23      A2
ATOM 1000 C   MET 127      60.847 71.131 -15.599 1.00 82.18      A2
ATOM 1001 OT1 MET 127      60.116 71.958 -16.142 1.00 81.86      A2
ATOM 1002 OT2 MET 127      61.267 71.285 -14.446 1.00 84.04      A2
ATOM 1003 CB  MET 138      39.323 80.595  -4.492 1.00 59.39      A1
ATOM 1004 CG  MET 138      40.123 79.298  -4.421 1.00 57.97      A1
ATOM 1005 SD  MET 138      40.561 78.973  -6.145 1.00 60.85      A3
ATOM 1006 CE  MET 138      41.129 77.310  -6.351 1.00 61.48      A1
ATOM 1007 C   MET 138      37.021 81.072  -5.454 1.00 60.26      A1
ATOM 1008 O   MET 138      36.832 82.262  -5.181 1.00 62.98      A1
ATOM 1009 HT1 MET 138      38.497 82.600  -6.075 1.00  0.00      A1
ATOM 1010 HT2 MET 138      38.313 81.757  -7.529 1.00  0.00      A1
ATOM 1011 N   MET 138      38.839 81.784  -6.639 1.00 60.49      A1
ATOM 1012 HT3 MET 138      39.865 81.816  -6.768 1.00  0.00      A1
ATOM 1013 CA  MET 138      38.445 80.672  -5.787 1.00 60.51      A1
ATOM 1014 N   PRO 139      35.995 80.242  -5.612 1.00 57.82      A1
ATOM 1015 CD  PRO 139      36.028 79.060  -6.448 1.00 58.10      A1
ATOM 1016 CA  PRO 139      34.654 80.538  -5.142 1.00 54.67      A1
ATOM 1017 CB  PRO 139      33.870 79.323  -5.525 1.00 54.54      A1
ATOM 1018 CG  PRO 139      34.945 78.290  -5.755 1.00 58.26      A1
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FIGURE 5

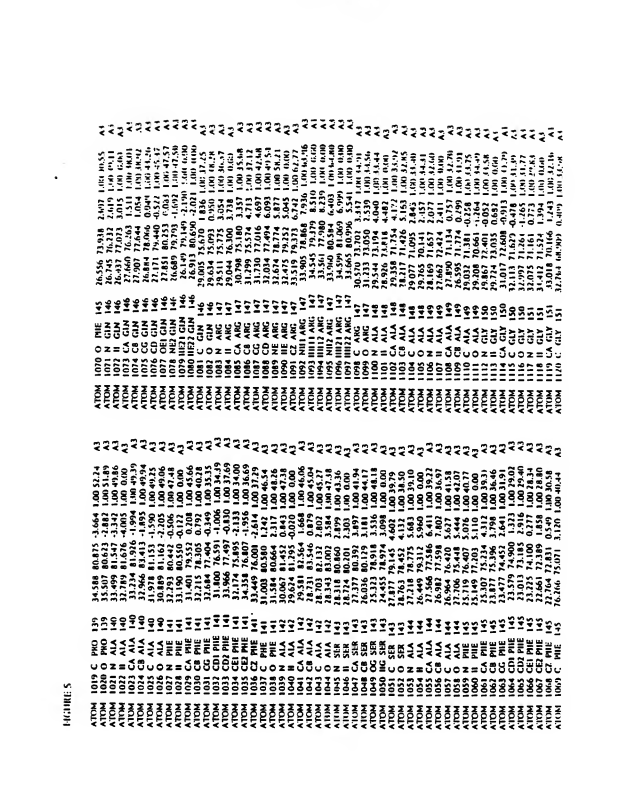

FIGURE 5

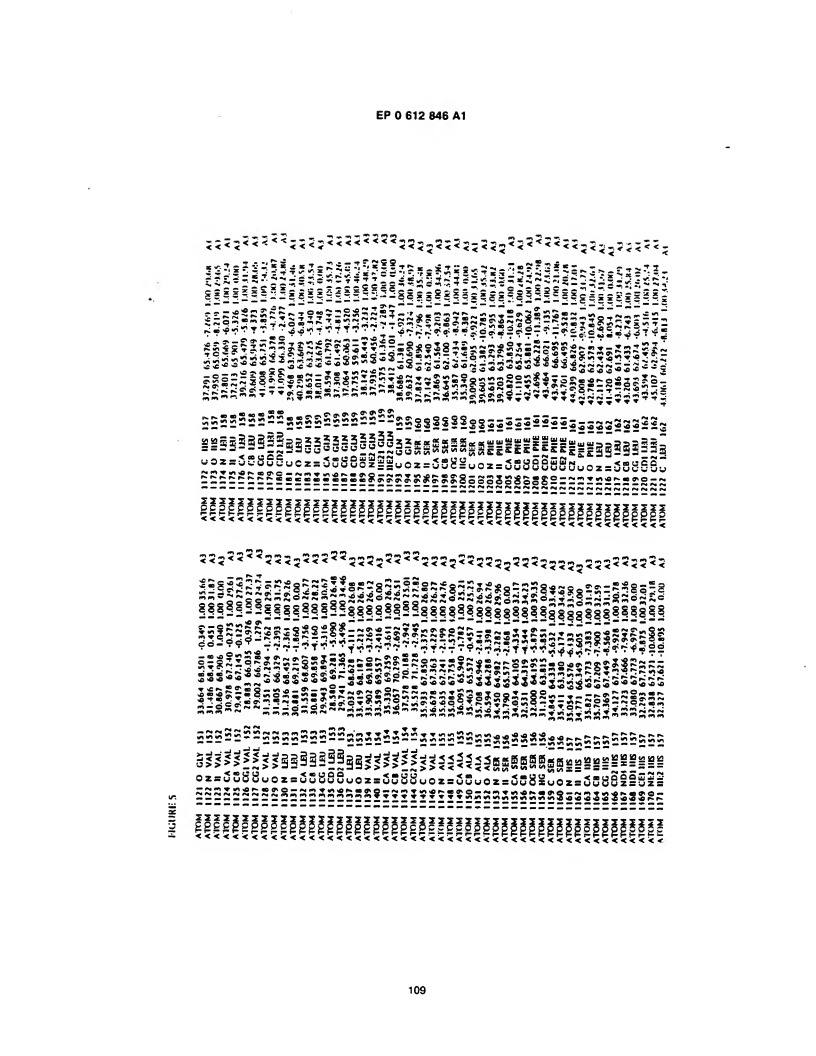

FIGURE 5

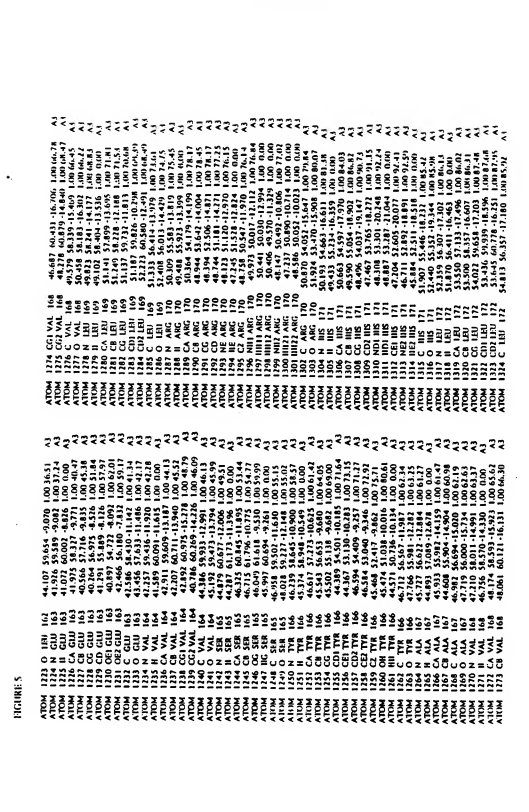

FIGURE 5

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ATOM  1325 O  LEU  172      55.896  56.660 -17.692  1.00 86.23      A3
ATOM  1326 N  ALA  173      54.733  55.383 -16.282  1.00 85.49      A3
ATOM  1327 H  ALA  173      53.899  55.276 -15.769  1.00  0.00      A3
ATOM  1328 CA ALA  173      55.856  54.497 -16.087  1.00 85.65      A3
ATOM  1329 CB ALA  173      56.602  54.859 -14.809  1.00 85.01      A3
ATOM  1330 C  ALA  173      55.330  53.073 -16.008  1.00 86.54      A3
ATOM  1331 OT1 ALA  173      55.585  52.347 -16.971  1.00 87.21      A3
ATOM  1332 OT2 ALA  173      54.650  52.707 -15.036  1.00 87.31      A3
ATOM  1333 CB LEU  210      45.234  42.591  25.453  1.00 52.47      B1
ATOM  1334 CG LEU  210      43.799  42.058  25.547  1.00 51.68      B1
ATOM  1335 CD1 LEU  210      43.123  42.362  26.804  1.00 53.37      B1
ATOM  1336 CD2 LEU  210      43.050  42.453  24.303  1.00 51.37      B1
ATOM  1337 C  LEU  210      46.770  44.374  24.596  1.00 50.98      B1
ATOM  1338 O  LEU  210      46.475  45.267  23.790  1.00 51.76      B1
ATOM  1339 HT1 LEU  210      44.382  44.922  24.421  1.00  0.00      B1
ATOM  1340 HT2 LEU  210      45.157  45.974  25.414  1.00  0.00      B1
ATOM  1341 N  LEU  210      44.705  45.041  25.406  1.00 53.59      B1
ATOM  1342 HT3 LEU  210      43.855  45.012  25.997  1.00  0.00      B1
ATOM  1343 CA LEU  210      45.730  44.038  25.676  1.00 52.35      B1
ATOM  1344 N  PRO  211      47.974  43.825  24.494  1.00 49.35      B1
ATOM  1345 CD PRO  211      48.621  43.074  25.532  1.00 49.52      B1
ATOM  1346 CA PRO  211      48.895  44.191  23.419  1.00 49.04      B1
ATOM  1347 CB PRO  211      50.209  43.571  23.865  1.00 49.02      B1
ATOM  1348 CG PRO  211      49.794  42.438  24.783  1.00 49.77      B1
ATOM  1349 C  PRO  211      48.543  43.864  21.965  1.00 48.03      B1
ATOM  1350 O  PRO  211      47.872  42.896  21.621  1.00 49.05      B1
ATOM  1351 N  GLN  212      49.032  44.675  21.051  1.00 46.52      B1
ATOM  1352 H  GLN  212      49.506  45.478  21.349  1.00  0.00      B1
ATOM  1353 CA GLN  212      48.839  44.461  19.641  1.00 45.47      B1
ATOM  1354 CB GLN  212      49.533  45.522  18.849  1.00 46.81      B1
ATOM  1355 CG GLN  212      48.482  46.139  17.999  1.00 49.55      B1
ATOM  1356 CD GLN  212      49.024  46.703  16.709  1.00 54.21      B1
ATOM  1357 OE1 GLN  212      48.429  47.672  16.232  1.00 57.72      B1
ATOM  1358 NE2 GLN  212      50.086  46.176  16.074  1.00 52.39      B1
ATOM  1359 HE21 GLN  212      50.530  45.383  16.430  1.00  0.00      B1
ATOM  1360 HE22 GLN  212      50.341  46.625  15.244  1.00  0.00      B1
ATOM  1361 C  GLN  212      49.390  43.133  19.185  1.00 44.79      B1
ATOM  1362 O  GLN  212      48.959  42.520  18.208  1.00 44.01      B1
ATOM  1363 N  SER  213      50.401  42.671  19.893  1.00 44.72      B1
ATOM  1364 H  SER  213      50.710  43.115  20.698  1.00  0.00      B1
ATOM  1365 CA SER  213      51.025  41.424  19.521  1.00 43.76      B1
ATOM  1366 CB SER  213      52.220  41.124  20.354  1.00 45.29      B1
ATOM  1367 OG SER  213      51.802  41.455  21.681  1.00 52.50      B1
ATOM  1368 HG SER  213      52.479  41.127  22.288  1.00  0.00      B1
ATOM  1369 C  SER  213      50.014  40.376  19.784  1.00 40.92      B1
ATOM  1370 O  SER  213      49.964  39.492  18.947  1.00 43.32      B1
ATOM  1371 N  PHE  214      49.242  40.571  20.876  1.00 38.86      B1
ATOM  1372 H  PHE  214      49.414  41.370  21.410  1.00  0.00      B1
ATOM  1373 CA PHE  214      48.210  39.664  21.336  1.00 37.40      B1
ATOM  1374 CB PHE  214      47.568  40.064  22.634  1.00 37.45      B1
ATOM  1375 CG PHE  214      46.494  39.080  23.035  1.00 41.01      B1
ATOM  1376 CD1 PHE  214      45.176  39.459  23.044  1.00 42.77      B1
ATOM  1377 CD2 PHE  214      46.818  37.794  23.400  1.00 42.02      B1
ATOM  1378 CE1 PHE  214      44.197  38.554  23.423  1.00 41.82      B1
ATOM  1379 CE2 PHE  214      45.834  36.898  23.776  1.00 41.70      B1
ATOM  1380 CZ PHE  214      44.519  37.277  23.791  1.00 41.05      B1
ATOM  1381 C  PHE  214      47.109  39.656  20.321  1.00 36.54      B1
ATOM  1382 O  PHE  214      46.735  38.566  19.889  1.00 37.99      B1
ATOM  1383 N  LEU  215      46.616  40.812  19.893  1.00 34.27      B1
ATOM  1384 H  LEU  215      47.008  41.642  20.238  1.00  0.00      B1
ATOM  1385 CA LEU  215      45.504  40.864  18.966  1.00 30.38      B1
ATOM  1386 CB LEU  215      45.099  42.282  18.701  1.00 31.82      B1
ATOM  1387 CG LEU  215      43.857  42.530  17.893  1.00 32.78      B1
ATOM  1388 CD1 LEU  215      42.727  41.963  18.737  1.00 34.95      B1
ATOM  1389 CD2 LEU  215      43.688  44.011  17.508  1.00 28.93      B1
ATOM  1390 C  LEU  215      45.811  40.232  17.648  1.00 29.57      B1
ATOM  1391 O  LEU  215      44.922  39.632  17.055  1.00 31.48      B1
ATOM  1392 N  LEU  216      47.031  40.379  17.155  1.00 29.44      B1
ATOM  1393 H  LEU  216      47.677  40.935  17.646  1.00  0.00      B1
ATOM  1394 CA LEU  216      47.465  39.790  15.893  1.00 29.89      B1
ATOM  1395 CB LEU  216      48.791  40.450  15.472  1.00 28.61      B1
ATOM  1396 CG LEU  216      48.682  41.877  14.939  1.00 26.83      B1
ATOM  1397 CD1 LEU  216      49.925  42.558  15.344  1.00 28.57      B1
ATOM  1398 CD2 LEU  216      48.446  41.950  13.452  1.00 24.09      B1
ATOM  1399 C  LEU  216      47.613  38.274  16.062  1.00 31.25      B1
ATOM  1400 O  LEU  216      47.328  37.514  15.138  1.00 29.70      B1
ATOM  1401 N  LYS  217      47.995  37.836  17.261  1.00 32.50      B1
ATOM  1402 H  LYS  217      48.305  38.482  17.926  1.00  0.00      B1
ATOM  1403 CA LYS  217      48.067  36.439  17.599  1.00 34.90      B1
ATOM  1404 CB LYS  217      48.645  36.280  19.002  1.00 38.07      B1
ATOM  1405 CG LYS  217      49.394  34.978  19.109  1.00 45.25      B1
ATOM  1406 CD LYS  217      49.714  34.491  20.521  1.00 52.17      B1
ATOM  1407 CE LYS  217      50.229  33.024  20.297  1.00 53.01      B1
ATOM  1408 NZ LYS  217      50.213  32.135  21.467  1.00 62.10      B1
ATOM  1409 1HZ1 LYS  217      49.239  32.056  21.824  1.00  0.00      B1
ATOM  1410 HZ2 LYS  217      50.830  32.515  22.214  1.00  0.00      B1
ATOM  1411 HZ3 LYS  217      50.554  31.195  21.179  1.00  0.00      B1
ATOM  1412 C  LYS  217      46.617  35.950  17.546  1.00 36.77      B1
ATOM  1413 O  LYS  217      46.311  34.933  16.886  1.00 39.58      B1
ATOM  1414 N  CYS  218      45.664  36.638  18.177  1.00 34.86      B1
ATOM  1415 H  CYS  218      45.907  37.388  18.751  1.00  0.00      B1
ATOM  1416 CA CYS  218      44.277  36.238  18.076  1.00 33.61      B1
ATOM  1417 CB CYS  218      43.430  37.175  18.846  1.00 33.71      B1
ATOM  1418 SG CYS  218      43.856  36.710  20.515  1.00 35.92      B1
ATOM  1419 C  CYS  218      43.766  36.189  16.652  1.00 32.89      B1
ATOM  1420 O  CYS  218      43.155  35.169  16.323  1.00 34.71      B1
ATOM  1421 N  LEU  219      44.035  37.169  15.777  1.00 29.52      B1
ATOM  1422 H  LEU  219      44.512  37.960  16.104  1.00  0.00      B1
ATOM  1423 CA LEU  219      43.614  37.119  14.391  1.00 27.44      B1
ATOM  1424 CB LEU  219      44.116  38.412  13.727  1.00 26.24      B1
ATOM  1425 CG LEU  219      43.884  38.768  12.241  1.00 25.07      B1
ATOM  1426 CD1 LEU  219      42.402  38.975  11.996  1.00 26.24      B1
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EP 0 612 846 A1

FIGURE 5

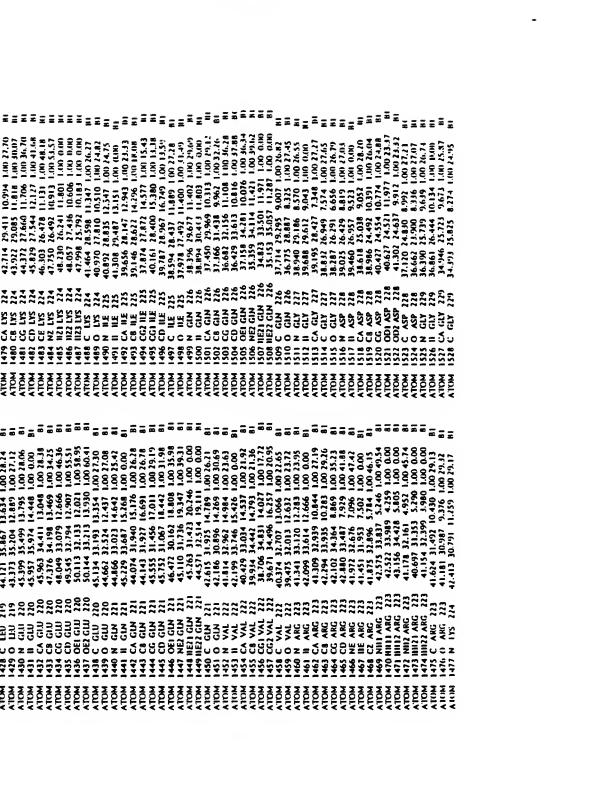

FIGURE 5

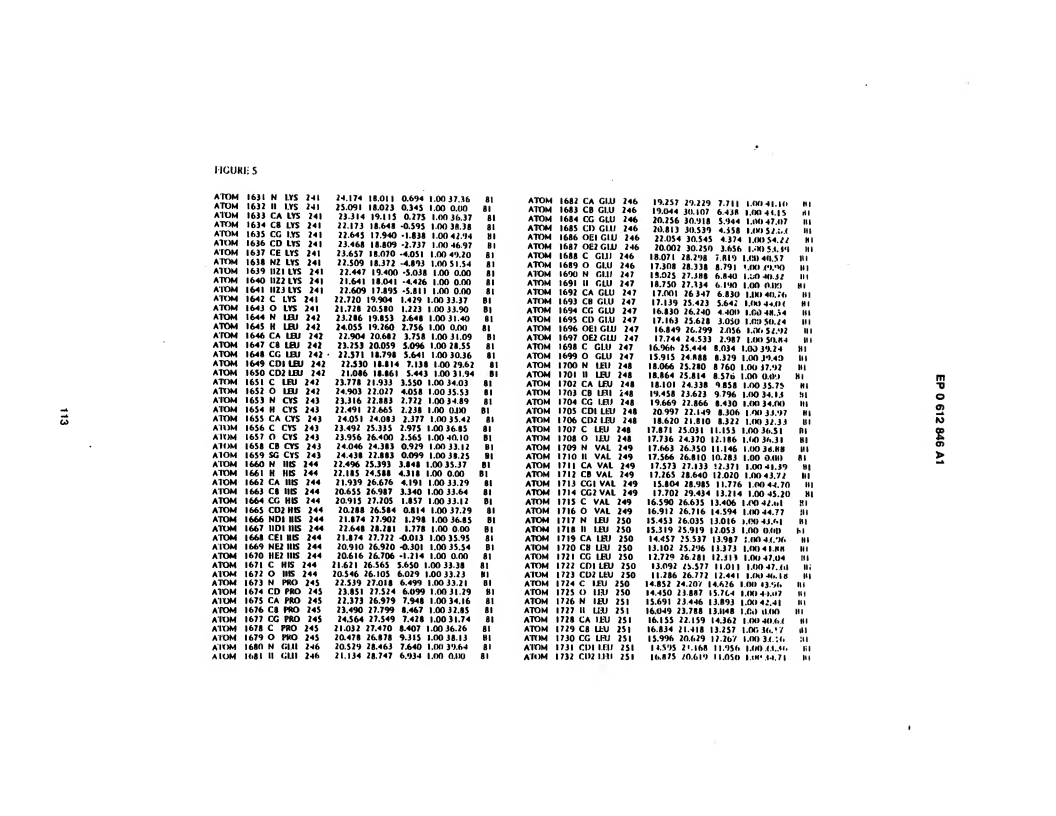

FIGURE 5

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ATOM  1733 C  LEU 251    17.104  22.372  15.493  1.00 42.78      B1
ATOM  1734 O  LEU 251    17.124  21.554  16.395  1.00 45.44      B1
ATOM  1735 N  GLY 252    17.826  23.477  15.610  1.00 44.86      B1
ATOM  1736 H  GLY 252    17.750  24.160  14.910  1.00  0.00      B1
ATOM  1737 CA GLY 252    18.734  23.711  16.719  1.00 46.68      B1
ATOM  1738 C  GLY 252    18.071  23.596  18.067  1.00 49.18      B1
ATOM  1739 O  GLY 252    18.709  23.318  19.077  1.00 49.23      B1
ATOM  1740 N  HIS 253    16.756  23.787  18.046  1.00 53.74      B1
ATOM  1741 H  HIS 253    16.358  24.055  17.190  1.00  0.00      B1
ATOM  1742 CA HIS 253    15.859  23.649  19.197  1.00 57.46      B1
ATOM  1743 CB HIS 253    14.468  24.157  18.764  1.00 62.93      B1
ATOM  1744 CG HIS 253    13.212  23.813  19.577  1.00 68.75      B1
ATOM  1745 CD2 HIS 253    12.031  24.529  19.414  1.00 71.00      B1
ATOM  1746 ND1 HIS 253    12.980  22.854  20.479  1.00 70.67      B1
ATOM  1747 HD1 HIS 253    13.627  22.193  20.830  1.00  0.00      B1
ATOM  1748 CE1 HIS 253    11.723  22.966  20.845  1.00 73.40      B1
ATOM  1749 HE1 HIS 253    11.356  23.873  20.204  1.00 72.91      B1
ATOM  1750 NE2 HIS 253    10.918  24.260  20.311  1.00  0.00      B1
ATOM  1751 C  HIS 253    15.771  22.209  19.691  1.00 56.06      B1
ATOM  1752 O  HIS 253    15.880  21.827  20.857  1.00 56.17      B1
ATOM  1753 N  SER 254    15.395  21.435  18.724  1.00 53.46      B1
ATOM  1754 H  SER 254    15.278  21.783  17.813  1.00  0.00      B1
ATOM  1755 CA SER 254    15.173  20.034  18.898  1.00 52.61      B1
ATOM  1756 CB SER 254    14.613  19.595  17.576  1.00 53.04      B1
ATOM  1757 OG SER 254    13.793  20.686  17.158  1.00 56.04      B1
ATOM  1758 HG SER 254    13.369  20.467  16.319  1.00  0.00      B1
ATOM  1759 C  SER 254    16.512  19.386  19.275  1.00 51.48      B1
ATOM  1760 O  SER 254    16.596  18.639  20.245  1.00 51.90      B1
ATOM  1761 N  LEU 255    17.577  19.790  18.562  1.00 49.31      B1
ATOM  1762 H  LEU 255    17.430  20.480  17.889  1.00  0.00      B1
ATOM  1763 CA LEU 255    18.913  19.272  18.723  1.00 46.02      B1
ATOM  1764 CB LEU 255    19.706  19.723  17.537  1.00 44.66      B1
ATOM  1765 CG LEU 255    19.362  18.968  16.274  1.00 44.51      B1
ATOM  1766 CD1 LEU 255    19.810  19.679  15.006  1.00 43.16      B1
ATOM  1767 CD2 LEU 255    19.969  17.604  16.456  1.00 44.67      B1
ATOM  1768 C  LEU 255    19.536  19.718  20.012  1.00 46.56      B1
ATOM  1769 O  LEU 255    20.565  19.174  20.440  1.00 46.82      B1
ATOM  1770 N  GLY 256    18.918  20.759  20.581  1.00 45.93      B1
ATOM  1771 H  GLY 256    18.210  21.225  20.101  1.00  0.00      B1
ATOM  1772 CA GLY 256    19.277  21.273  21.890  1.00 46.68      B1
ATOM  1773 C  GLY 256    20.669  21.866  21.970  1.00 47.28      B1
ATOM  1774 O  GLY 256    21.273  21.844  23.056  1.00 49.64      B1
ATOM  1775 N  ILE 257    21.143  22.441  20.849  1.00 45.74      B1
ATOM  1776 H  ILE 257    20.497  22.549  20.128  1.00  0.00      B1
ATOM  1777 CA ILE 257    22.481  23.017  20.726  1.00 43.64      B1
ATOM  1778 CB ILE 257    22.684  23.363  19.257  1.00 42.54      B1
ATOM  1779 CG2 ILE 257    23.988  24.110  19.073  1.00 41.05      B1
ATOM  1780 CG1 ILE 257    22.694  22.088  18.437  1.00 40.55      B1
ATOM  1781 CD ILE 257    22.452  22.468  16.970  1.00 39.49      B1
ATOM  1782 C  ILE 257    22.559  24.246  21.616  1.00 43.27      B1
ATOM  1783 O  ILE 257    21.706  25.110  21.450  1.00 43.22      B1
ATOM  1784 N  PRO 258    23.441  24.392  22.608  1.00 43.35      B1
ATOM  1785 CD PRO 258    24.333  23.321  23.296  1.00 43.29      B1
ATOM  1786 CA PRO 258    23.559  25.616  23.360  1.00 43.82      B1
ATOM  1787 CB PRO 258    24.295  25.236  24.612  1.00 41.97      B1
ATOM  1788 CG PRO 258    25.107  24.064  24.186  1.00 42.79      B1
ATOM  1789 C  PRO 258    24.252  26.703  22.555  1.00 46.36      B1
ATOM  1790 O  PRO 258    24.983  26.513  21.560  1.00 46.59      B1
ATOM  1791 N  TRP 259    23.996  27.887  23.106  1.00 46.75      B1
ATOM  1792 H  TRP 259    23.588  27.921  23.994  1.00  0.00      B1
ATOM  1793 CA TRP 259    24.427  29.143  22.517  1.00 45.77      B1
ATOM  1794 CB TRP 259    23.213  30.071  22.397  1.00 46.64      B1
ATOM  1795 CG TRP 259    23.556  31.372  21.749  1.00 47.51      B1
ATOM  1796 CD2 TRP 259    23.860  31.525  20.430  1.00 47.84      B1
ATOM  1797 CE2 TRP 259    24.154  32.808  20.392  1.00 48.47      B1
ATOM  1798 CE3 TRP 259    23.940  30.745  19.290  1.00 47.39      B1
ATOM  1799 CD1 TRP 259    23.639  32.520  22.493  1.00 48.60      B1
ATOM  1800 NE1 TRP 259    24.013  33.421  21.628  1.00 48.17      B1
ATOM  1801 HE1 TRP 259    24.224  34.344  21.870  1.00  0.00      B1
ATOM  1802 CZ2 TRP 259    24.531  33.486  19.195  1.00 47.40      B1
ATOM  1803 CZ3 TRP 259    24.317  31.344  18.097  1.00 49.07      B1
ATOM  1804 CH2 TRP 259    24.613  32.706  18.050  1.00 49.12      B1
ATOM  1805 C  TRP 259    25.459  29.727  23.440  1.00 44.01      B1
ATOM  1806 O  TRP 259    25.340  29.664  24.671  1.00 43.25      B1
ATOM  1807 N  ALA 260    26.469  30.247  22.777  1.00 43.01      B1
ATOM  1808 H  ALA 260    26.523  30.198  21.796  1.00  0.00      B1
ATOM  1809 CA ALA 260    27.593  30.973  23.482  1.00 43.48      B1
ATOM  1810 CB ALA 260    28.874  30.549  22.969  1.00 43.34      B1
ATOM  1811 C  ALA 260    27.249  32.486  23.216  1.00 43.41      B1
ATOM  1812 O  ALA 260    27.315  32.946  22.054  1.00 40.55      B1
ATOM  1813 N  PRO 261    26.853  33.267  24.253  1.00 42.61      B1
ATOM  1814 CD PRO 261    26.527  32.807  25.606  1.00 42.13      B1
ATOM  1815 CA PRO 261    26.720  34.703  24.199  1.00 42.37      B1
ATOM  1816 CB PRO 261    25.778  34.987  25.335  1.00 41.46      B1
ATOM  1817 CG PRO 261    26.253  34.060  26.411  1.00 40.30      B1
ATOM  1818 C  PRO 261    28.087  35.369  24.311  1.00 42.22      B1
ATOM  1819 O  PRO 261    28.988  34.956  25.037  1.00 38.82      B1
ATOM  1820 N  LEU 262    28.234  36.403  23.486  1.00 45.20      B1
ATOM  1821 H  LEU 262    27.513  36.610  22.853  1.00  0.00      B1
ATOM  1822 CA LEU 262    29.434  37.210  23.498  1.00 46.50      B1
ATOM  1823 CB LEU 262    30.531  36.609  22.610  1.00 45.09      B1
ATOM  1824 CG LEU 262    31.903  37.157  22.964  1.00 42.55      B1
ATOM  1825 CD1 LEU 262    32.344  36.695  24.338  1.00 41.52      B1
ATOM  1826 CD2 LEU 262    32.850  36.730  21.900  1.00 44.21      B1
ATOM  1827 C  LEU 262    29.154  38.628  23.035  1.00 48.56      B1
ATOM  1828 O  LEU 262    29.633  39.470  23.790  1.00 48.23      B1
ATOM  1829 N  SER 263    28.388  38.956  21.960  1.00 51.11      B1
ATOM  1830 H  SER 263    27.982  38.242  21.427  1.00 0.00      B1
ATOM  1831 CA SER 263    28.127  40.339  21.494  1.00 55.19      B1
ATOM  1832 CB SER 263    26.871  40.511  20.612  1.00 57.17      B1
ATOM  1833 OG SER 263    26.498  39.411  19.776  1.00 64.12      B1
ATOM  1834 HG SER 263    26.093  38.741  20.336  1.00 0.00      B1
```

FIGURE 5

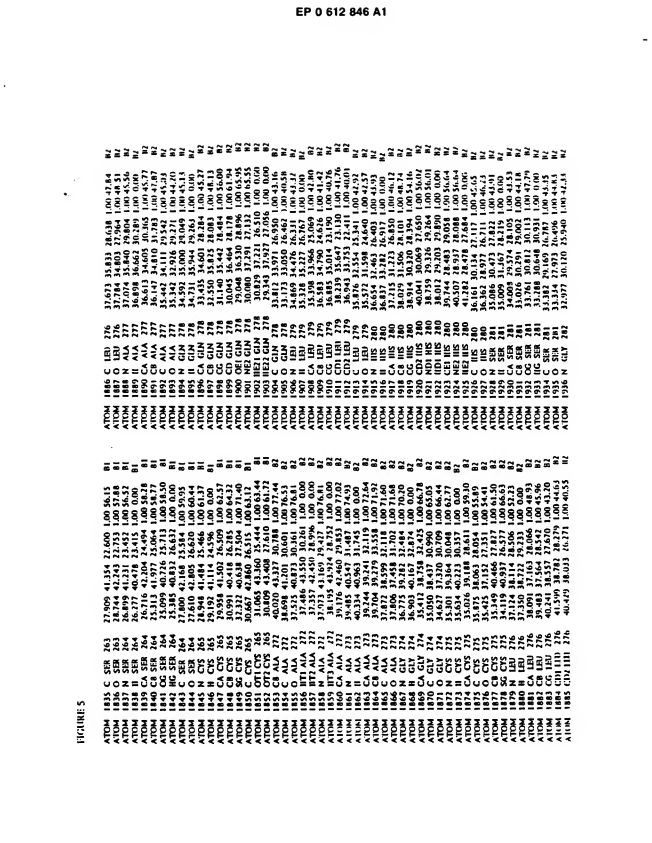
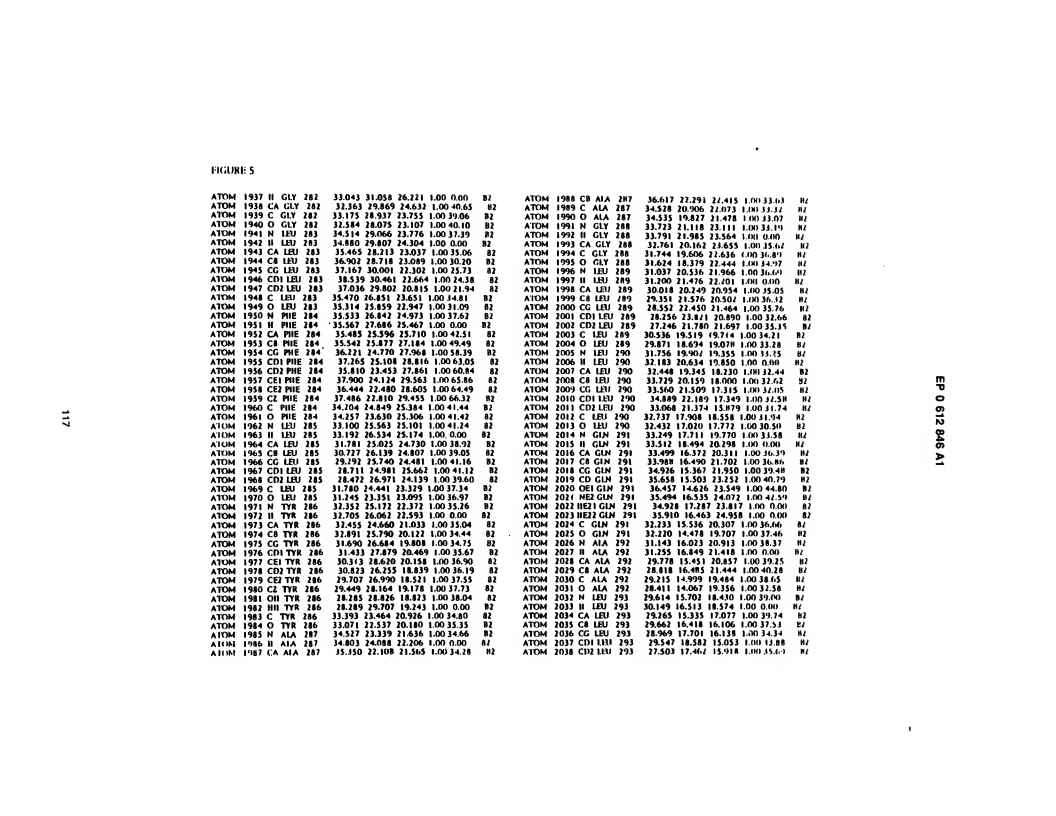


FIGURE 5



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ATOM  1937 H  GLY  282      33.043  31.058  26.221  1.00  0.00      B2
ATOM  1938 CA GLY  282      32.363  29.869  24.632  1.00 40.65      B2
ATOM  1939 C  GLY  282      33.175  28.937  23.755  1.00 39.06      B2
ATOM  1940 O  GLY  282      32.584  28.075  23.107  1.00 40.10      B2
ATOM  1941 N  LEU  283      34.514  29.066  23.776  1.00 37.39      B2
ATOM  1942 H  LEU  283      34.880  29.807  24.304  1.00  0.00      B2
ATOM  1943 CA LEU  283      35.465  28.213  23.037  1.00 35.06      B2
ATOM  1944 CB LEU  283      36.902  28.718  23.089  1.00 30.20      B2
ATOM  1945 CG LEU  283      37.167  30.001  22.302  1.00 25.73      B2
ATOM  1946 CD1 LEU  283      38.539  30.464  22.664  1.00 24.18      B2
ATOM  1947 CD2 LEU  283      37.036  29.802  20.815  1.00 21.94      B2
ATOM  1948 C  LEU  283      35.470  26.851  23.651  1.00 34.81      B2
ATOM  1949 O  LEU  283      35.314  25.859  22.947  1.00 31.09      B2
ATOM  1950 N  PHE  284      35.533  26.842  24.973  1.00 37.62      B2
ATOM  1951 H  PHE  284      35.567  27.686  25.467  1.00  0.00      B2
ATOM  1952 CA PHE  284      35.485  25.596  25.710  1.00 41.51      B2
ATOM  1953 CB PHE  284      35.542  25.877  27.184  1.00 49.49      B2
ATOM  1954 CG PHE  284      36.721  24.770  27.968  1.00 58.39      B2
ATOM  1955 CD1 PHE  284      37.265  25.108  28.816  1.00 63.05      B2
ATOM  1956 CD2 PHE  284      35.810  23.453  27.861  1.00 60.84      B2
ATOM  1957 CE1 PHE  284      37.900  24.124  29.563  1.00 65.86      B2
ATOM  1958 CE2 PHE  284      36.444  22.480  28.605  1.00 64.49      B2
ATOM  1959 CZ PHE  284      37.486  22.810  29.455  1.00 66.32      B2
ATOM  1960 C  PHE  284      34.204  24.849  25.384  1.00 41.44      B2
ATOM  1961 O  PHE  284      34.257  23.630  25.306  1.00 41.82      B2
ATOM  1962 N  LEU  285      33.100  25.563  25.101  1.00 41.24      B2
ATOM  1963 H  LEU  285      33.192  26.534  25.174  1.00  0.00      B2
ATOM  1964 CA LEU  285      31.781  25.025  24.730  1.00 38.92      B2
ATOM  1965 CB LEU  285      30.727  26.139  24.807  1.00 39.05      B2
ATOM  1966 CG LEU  285      29.392  25.748  24.148  1.00 41.16      B2
ATOM  1967 CD1 LEU  285      28.711  24.981  25.662  1.00 41.12      B2
ATOM  1968 CD2 LEU  285      28.472  26.971  24.139  1.00 39.60      B2
ATOM  1969 C  LEU  285      31.780  24.441  23.329  1.00 37.34      B2
ATOM  1970 O  LEU  285      31.245  23.351  23.095  1.00 36.97      B2
ATOM  1971 N  TYR  286      32.352  25.172  22.372  1.00 35.26      B2
ATOM  1972 H  TYR  286      32.705  26.062  22.593  1.00 0.00       B2
ATOM  1973 CA TYR  286      32.455  24.660  21.033  1.00 35.04      B2
ATOM  1974 CB TYR  286      32.891  25.790  20.122  1.00 34.44      B2
ATOM  1975 CG TYR  286      31.690  26.644  19.808  1.00 34.75      B2
ATOM  1976 CD1 TYR  286      31.433  27.879  20.469  1.00 35.67      B2
ATOM  1977 CE1 TYR  286      30.313  28.620  20.158  1.00 36.90      B2
ATOM  1978 CD2 TYR  286      30.822  26.215  18.859  1.00 36.19      B2
ATOM  1979 CE2 TYR  286      29.707  26.990  18.521  1.00 37.55      B2
ATOM  1980 CZ TYR  286      29.449  28.164  19.178  1.00 37.73      B2
ATOM  1981 OH TYR  286      28.285  28.826  18.823  1.00 38.04      B2
ATOM  1982 HH TYR  286      28.289  29.707  19.243  1.00  0.00      B2
ATOM  1983 C  TYR  286      33.393  23.464  20.976  1.00 34.40      B2
ATOM  1984 O  TYR  286      33.071  22.537  20.180  1.00 35.35      B2
ATOM  1985 N  ALA  287      34.527  23.339  21.636  1.00 34.66      B2
ATOM  1986 H  ALA  287      34.803  24.088  22.206  1.00  0.00      B2
ATOM  1987 CA ALA  287      35.350  22.108  21.565  1.00 34.28      B2
ATOM  1988 CB ALA  287      36.617  22.291  22.415  1.00 33.63      B2
ATOM  1989 C  ALA  287      34.528  20.906  21.973  1.00 31.32      B2
ATOM  1990 O  ALA  287      34.535  19.827  21.478  1.00 33.07      B2
ATOM  1991 N  GLY  288      33.723  21.118  23.111  1.00 33.19      B2
ATOM  1992 H  GLY  288      33.791  21.985  23.564  1.00  0.00      B2
ATOM  1993 CA GLY  288      32.761  20.162  23.655  1.00 35.64      B2
ATOM  1994 C  GLY  288      31.744  19.606  22.636  1.00 36.83      B2
ATOM  1995 O  GLY  288      31.624  18.379  22.444  1.00 34.97      B2
ATOM  1996 N  LEU  289      31.037  20.536  21.966  1.00 36.60      B2
ATOM  1997 H  LEU  289      31.200  21.476  22.201  1.00  0.00      B2
ATOM  1998 CA LEU  289      30.018  20.249  20.954  1.00 35.05      B2
ATOM  1999 CB LEU  289      29.351  21.576  20.502  1.00 36.32      B2
ATOM  2000 CG LEU  289      28.552  22.450  21.464  1.00 35.76      B2
ATOM  2001 CD1 LEU  289      28.256  23.821  20.890  1.00 32.66      B2
ATOM  2002 CD2 LEU  289      27.246  21.780  21.697  1.00 35.35      B2
ATOM  2003 C  LEU  289      30.536  19.519  19.714  1.00 34.21      B2
ATOM  2004 O  LEU  289      29.871  18.694  19.078  1.00 33.28      B2
ATOM  2005 N  LEU  290      31.756  19.903  19.355  1.00 33.35      B2
ATOM  2006 H  LEU  290      32.183  20.634  19.850  1.00  0.00      B2
ATOM  2007 CA LEU  290      32.448  19.345  18.230  1.00 32.44      B2
ATOM  2008 C8 LEU  290      33.729  20.159  18.000  1.00 32.62      B2
ATOM  2009 CG LEU  290      34.691  21.509  17.315  1.00 32.05      B2
ATOM  2010 CD1 LEU  290      34.889  22.189  17.349  1.00 31.58      B2
ATOM  2011 CD2 LEU  290      33.068  21.374  15.879  1.00 31.74      B2
ATOM  2012 C  LEU  290      31.737  18.908  18.558  1.00 31.94      B2
ATOM  2013 O  LEU  290      32.432  17.020  17.772  1.00 30.50      B2
ATOM  2014 N  GLN  291      33.249  17.711  19.770  1.00 33.58      B2
ATOM  2015 H  GLN  291      33.285  18.494  20.398  1.00  0.00      B2
ATOM  2016 CA GLN  291      33.499  16.372  20.311  1.00 36.39      B2
ATOM  2017 CB GLN  291      35.007  16.490  21.702  1.00 36.86      B2
ATOM  2018 CG GLN  291      34.926  15.367  21.950  1.00 39.48      B2
ATOM  2019 CD GLN  291      35.658  15.503  23.252  1.00 40.79      B2
ATOM  2020 OE1 GLN  291      36.457  14.626  23.549  1.00 40.40      B2
ATOM  2021 NE2 GLN  291      35.494  16.535  24.072  1.00 42.59      B2
ATOM  2022 HE21 GLN  291      34.928  17.287  23.817  1.00  0.00      B2
ATOM  2023 HE22 GLN  291      35.910  16.463  24.958  1.00  0.00      B2
ATOM  2024 C  GLN  291      32.233  15.536  20.307  1.00 36.66      B2
ATOM  2025 O  GLN  291      32.220  14.478  19.707  1.00 37.46      B2
ATOM  2026 N  ALA  292      31.143  16.023  20.913  1.00 38.37      B2
ATOM  2027 H  ALA  292      31.255  16.849  21.418  1.00  0.00      B2
ATOM  2028 CA ALA  292      29.778  15.451  20.857  1.00 39.25      B2
ATOM  2029 CB ALA  292      28.818  16.485  21.444  1.00 40.28      B2
ATOM  2030 C  ALA  292      29.215  14.999  19.484  1.00 38.65      B2
ATOM  2031 O  ALA  292      28.411  14.067  19.356  1.00 32.58      B2
ATOM  2032 N  LEU  293      29.614  15.702  18.430  1.00 39.00      B2
ATOM  2033 H  LEU  293      30.149  16.513  18.574  1.00  0.00      B2
ATOM  2034 CA LEU  293      29.265  15.335  17.077  1.00 39.74      B2
ATOM  2035 CB LEU  293      29.662  16.418  16.106  1.00 37.53      B2
ATOM  2036 CG LEU  293      28.969  17.701  16.138  1.00 34.84      B2
ATOM  2037 CD1 LEU  293      29.547  18.582  15.053  1.00 31.88      B2
ATOM  2038 CD2 LEU  293      27.503  17.462  15.918  1.00 35.63      B2
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EP 0 612 846 A1

FIGURE 5

FIGURE 5

FIGURE 5

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ATOM  2244 HG1 THR  317      46.489  30.441  21.389  1.00  0.00      B2
ATOM  2245 CG2 THR  317      46.109  33.566  22.156  1.00 34.30      B2
ATOM  2246  C  THR  317      45.338  32.597  24.832  1.00 39.30      B2
ATOM  2247  O  THR  317      45.941  33.378  25.583  1.00 40.17      B2
ATOM  2248  N  ILE  318      44.003  32.481  24.912  1.00 40.83      B2
ATOM  2249 CA  ILE  318      43.172  33.317  25.788  1.00 40.75      B2
ATOM  2250 CB  ILE  318      41.621  32.979  25.567  1.00 37.17      B2
ATOM  2251 CG2 ILE  318      40.742  33.706  26.545  1.00 34.29      B2
ATOM  2252 CG1 ILE  318      41.216  33.310  24.160  1.00 31.39      B2
ATOM  2253 CD  ILE  318      41.626  34.657  23.614  1.00 29.66      B2
ATOM  2254  C  ILE  318      43.624  33.019  27.217  1.00 42.43      B2
ATOM  2255  O  ILE  318      44.064  33.963  27.856  1.00 42.54      B2
ATOM  2256  N  TRP  319      43.662  31.784  27.744  1.00 44.17      B2
ATOM  2257  H  TRP  319      43.537  31.008  27.163  1.00  0.00      B2
ATOM  2258 CA  TRP  319      43.994  31.633  29.142  1.00 46.90      B2
ATOM  2259 CB  TRP  319      43.892  30.179  29.597  1.00 50.64      B2
ATOM  2260 CG  TRP  319      43.998  30.094  31.131  1.00 56.05      B2
ATOM  2261 CD2  TRP  319      43.005  30.397  32.038  1.00 58.61      B2
ATOM  2262 CE2  TRP  319      43.685  30.281  33.251  1.00 60.50      B2
ATOM  2263 CE3  TRP  319      41.668  30.740  32.005  1.00 60.12      B2
ATOM  2264 CD1  TRP  319      45.188  29.788  31.760  1.00 58.07      B2
ATOM  2265 NE1  TRP  319      44.968  29.921  33.042  1.00 60.07      B2
ATOM  2266 HE1  TRP  319      45.637  29.765  33.740  1.00  0.00      B2
ATOM  2267 CZ2  TRP  319      43.044  30.512  34.456  1.00 61.00      B2
ATOM  2268 CZ3  TRP  319      41.022  30.967  33.210  1.00 61.58      B2
ATOM  2269 CH2  TRP  319      41.704  30.854  34.417  1.00 62.04      B2
ATOM  2270  C  TRP  319      45.398  32.136  29.450  1.00 47.85      B2
ATOM  2271  O  TRP  319      45.635  32.772  30.490  1.00 47.99      B2
ATOM  2272  N  GLN  320      46.339  31.915  28.550  1.00 48.63      B2
ATOM  2273  H  GLN  320      46.091  31.482  27.708  1.00  0.00      B2
ATOM  2274 CA  GLN  320      47.706  32.319  28.767  1.00 49.45      B2
ATOM  2275 CB  GLN  320      48.567  31.988  27.589  1.00 51.44      B2
ATOM  2276 CG  GLN  320      48.828  30.494  27.444  1.00 55.03      B2
ATOM  2277 CD  GLN  320      49.958  30.349  26.438  1.00 60.17      B2
ATOM  2278 OE1  GLN  320      51.116  30.465  26.834  1.00 65.26      B2
ATOM  2279 NE2  GLN  320      49.771  30.145  25.131  1.00 59.97      B2
ATOM  2280 HE21  GLN  320      48.859  30.087  24.789  1.00  0.00      B2
ATOM  2281 HE22  GLN  320      50.582  30.083  24.590  1.00  0.00      B2
ATOM  2282  C  GLN  320      47.717  33.792  29.149  1.00 49.62      B2
ATOM  2283  O  GLN  320      48.251  34.209  29.987  1.00 49.91      B2
ATOM  2284  N  GLN  321      46.998  34.538  28.150  1.00 51.76      B2
ATOM  2285  H  GLN  321      46.535  34.102  27.403  1.00 55.03      B2
ATOM  2286 CA  GLN  321      46.837  35.988  28.278  1.00 52.08      B2
ATOM  2287 CB  GLN  321      46.015  36.571  27.151  1.00 49.72      B2
ATOM  2288 CG  GLN  321      45.873  38.058  27.166  1.00 51.19      B2
ATOM  2289 CD  GLN  321      47.211  38.781  27.201  1.00 53.13      B2
ATOM  2290 OE1  GLN  321      48.090  38.622  26.364  1.00 55.36      B2
ATOM  2291 NE2  GLN  321      47.468  39.618  28.177  1.00 53.21      B2
ATOM  2292 HE21  GLN  321      46.800  39.713  28.889  1.00  0.00      B2
ATOM  2293 HE22  GLN  321      48.338  40.057  28.168  1.00  0.00      B2

ATOM  2294  C  GLN  321      46.112  36.315  29.562  1.00 54.30      B2
ATOM  2295  O  GLN  321      46.293  37.422  30.058  1.00 54.39      B2
ATOM  2296  N  MET  322      45.269  35.441  30.117  1.00 54.50      B2
ATOM  2297  H  MET  322      45.098  34.592  29.662  1.00  0.00      B2
ATOM  2298 CA  MET  322      44.619  35.748  31.375  1.00 55.42      B2
ATOM  2299 CB  MET  322      43.595  34.690  31.713  1.00 52.91      B2
ATOM  2300 CG  MET  322      42.527  34.865  30.658  1.00 51.76      B2
ATOM  2301 SD  MET  322      40.861  34.428  31.189  1.00 54.19      B2
ATOM  2302 CE  MET  322      40.293  33.192  30.069  1.00 52.53      B2
ATOM  2303  C  MET  322      45.700  35.811  32.437  1.00 57.69      B2
ATOM  2304  O  MET  322      45.781  36.739  33.248  1.00 57.85      B2
ATOM  2305  N  GLU  323      46.652  34.900  32.319  1.00 60.28      B2
ATOM  2306  H  GLU  323      46.637  34.296  31.544  1.00  0.00      B2
ATOM  2307 CA  GLU  323      47.741  34.875  33.273  1.00 62.99      B2
ATOM  2308 CB  GLU  323      48.558  33.615  32.957  1.00 65.81      B2
ATOM  2309 CG  GLU  323      47.640  32.423  32.918  1.00 68.36      B2
ATOM  2310 CD  GLU  323      48.303  31.125  33.310  1.00 71.21      B2
ATOM  2311 OE1  GLU  323      47.651  30.364  34.044  1.00 71.19      B2
ATOM  2312 OE2  GLU  323      49.451  30.900  32.884  1.00 72.43      B2
ATOM  2313  C  GLU  323      48.648  36.124  33.418  1.00 63.96      B2
ATOM  2314 OT1  GLU  323      48.782  36.492  34.584  1.00 64.11      B2
ATOM  2315 OT2  GLU  323      49.169  36.725  32.449  1.00 62.96      B2
ATOM  2316 CB  MET  338      27.559  17.690  25.056  1.00 62.56      B3
ATOM  2317 CG  MET  338      28.087  18.862  24.222  1.00 63.85      B3
ATOM  2318 SD  MET  338      27.248  20.324  25.219  1.00 66.95      B3
ATOM  2319 CE  MET  338      27.328  21.252  25.515  1.00 65.30      B3
ATOM  2320  C  MET  338      24.988  17.301  25.122  1.00 57.55      B3
ATOM  2321  O  MET  338      24.412  16.347  25.667  1.00 56.47      B3
ATOM  2322 HT1  MET  338      26.255  16.010  26.594  1.00  0.00      B3
ATOM  2323 HT2  MET  338      25.375  17.061  27.300  1.00  0.00      B3
ATOM  2324  N  MET  338      26.786  16.971  27.009  1.00 61.55      B3
ATOM  2325 HT3  MET  338      27.108  17.107  27.620  1.00  0.00      B3
ATOM  2326 CA  MET  338      26.226  17.853  25.851  1.00 60.35      B3
ATOM  2327  N  PRO  339      24.493  17.830  23.998  1.00 55.58      B3
ATOM  2328 CD  PRO  339      24.914  19.075  23.375  1.00 54.49      B3
ATOM  2329 CA  PRO  339      23.453  17.226  23.164  1.00 54.62      B3
ATOM  2330 CB  PRO  339      23.463  18.098  21.903  1.00 53.52      B3
ATOM  2331 CG  PRO  339      24.845  18.711  21.909  1.00 53.04      B3
ATOM  2332  C  PRO  339      23.666  15.748  22.881  1.00 53.61      B3
ATOM  2333  O  PRO  339      24.730  15.222  23.169  1.00 53.35      B3
ATOM  2334  N  ALA  340      22.704  15.045  22.333  1.00 54.32      B3
ATOM  2335  H  ALA  340      21.844  15.460  22.111  1.00  0.00      B3
ATOM  2336 CA  ALA  340      22.909  13.651  21.968  1.00 56.04      B3
ATOM  2337 CB  ALA  340      21.867  12.713  22.625  1.00 57.60      B3
ATOM  2338  C  ALA  340      22.617  13.713  20.495  1.00 55.61      B3
ATOM  2339  O  ALA  340      21.426  13.783  20.196  1.00 58.64      B3
ATOM  2340  N  PHE  341      23.516  13.734  19.514  1.00 53.34      B3
ATOM  2341  H  PHE  341      24.472  13.607  19.685  1.00  0.00      B3
ATOM  2342 CA  PHE  341      23.016  13.900  18.158  1.00 49.92      B3
ATOM  2343 CB  PHE  341      24.050  14.541  17.244  1.00 48.16      B3
ATOM  2344 CG  PHE  341      24.382  15.940  17.658  1.00 45.00      B3
```

FIGURE 5

```
ATOM  2345 CD1 PHE  341      23.510  16.923  17.359  1.00 43.44      B3
ATOM  2346 CD2 PHE  341      25.527  16.175  18.388  1.00 47.03      B3
ATOM  2347 CE1 PHE  341      23.812  18.172  17.831  1.00 49.15      B3
ATOM  2348 CE2 PHE  341      25.827  17.426  18.862  1.00 47.86      B3
ATOM  2349 CZ  PHE  341      24.952  18.437  18.580  1.00 48.36      B3
ATOM  2350 C   PHE  341      22.684  12.510  17.672  1.00 49.56      B3
ATOM  2351 O   PHE  341      23.309  11.938  16.781  1.00 51.46      B3
ATOM  2352 N   ALA  342      21.625  11.985  18.245  1.00 47.40      B3
ATOM  2353 H   ALA  342      21.026  12.585  18.741  1.00  0.00      B3
ATOM  2354 CA  ALA  342      21.167  10.650  17.997  1.00 46.11      B3
ATOM  2355 CB  ALA  342      19.874  10.531  18.804  1.00 47.10      B3
ATOM  2356 C   ALA  342      20.962  10.149  16.556  1.00 44.37      B3
ATOM  2357 O   ALA  342      20.138   9.247  16.418  1.00 45.65      B3
ATOM  2358 N   SER  343      21.537  10.573  15.423  1.00 41.37      B3
ATOM  2359 H   SER  343      22.191  11.301  15.428  1.00  0.00      B3
ATOM  2360 CA  SER  343      21.274   9.923  14.145  1.00 38.80      B3
ATOM  2361 CB  SER  343      19.842  10.138  13.656  1.00 38.79      B3
ATOM  2362 OG  SER  343      19.205  11.300  14.182  1.00 37.75      B3
ATOM  2363 HG  SER  343      18.963  11.059  15.092  1.00  0.00      B3
ATOM  2364 C   SER  343      22.172  10.467  13.048  1.00 38.22      B3
ATOM  2365 O   SER  343      22.810  11.471  13.382  1.00 38.30      B3
ATOM  2366 N   ALA  344      22.206   9.845  11.888  1.00 36.73      B3
ATOM  2367 H   ALA  344      21.762   8.978  11.805  1.00  0.00      B3
ATOM  2368 CA  ALA  344      22.914  10.384  10.715  1.00 38.09      B3
ATOM  2369 CB  ALA  344      22.583   9.640   9.427  1.00 36.78      B3
ATOM  2370 C   ALA  344      22.472  11.842  10.496  1.00 37.72      B3
ATOM  2371 O   ALA  344      23.271  12.765  10.676  1.00 38.42      B3
ATOM  2372 N   PHE  345      21.194  12.042  10.163  1.00 36.10      B3
ATOM  2373 H   PHE  345      20.668  11.298   9.811  1.00  0.00      B3
ATOM  2374 CA  PHE  345      20.564  13.338  10.195  1.00 34.69      B3
ATOM  2375 CB  PHE  345      19.040  13.254  10.128  1.00 33.24      B3
ATOM  2376 CG  PHE  345      18.462  14.656   9.918  1.00 31.72      B3
ATOM  2377 CD1 PHE  345      17.715  15.273  10.905  1.00 26.64      B3
ATOM  2378 CD2 PHE  345      18.767  15.343   8.745  1.00 29.99      B3
ATOM  2379 CE1 PHE  345      17.284  16.503  10.682  1.00 33.56      B3
ATOM  2380 CE2 PHE  345      18.333  16.619   8.537  1.00 30.81      B3
ATOM  2381 CZ  PHE  345      17.581  17.201   9.520  1.00 31.44      B3
ATOM  2382 C   PHE  345      20.888  14.145  11.458  1.00 35.02      B3
ATOM  2383 O   PHE  345      21.246  15.319  11.292  1.00 37.81      B3
ATOM  2384 N   GLN  346      20.814  13.688  12.691  1.00 32.53      B3
ATOM  2385 H   GLN  346      20.516  12.778  12.894  1.00  0.00      B3
ATOM  2386 CA  GLN  346      21.156  14.586  13.758  1.00 33.46      B3
ATOM  2387 CB  GLN  346      20.899  13.935  15.061  1.00 33.80      B3
ATOM  2388 CG  GLN  346      19.459  14.284  15.174  1.00 35.68      B3
ATOM  2389 CD  GLN  346      18.788  13.658  16.344  1.00 38.48      B3
ATOM  2390 OE1 GLN  346      19.358  13.328  17.374  1.00 41.78      B3
ATOM  2391 NE2 GLN  346      17.508  13.463  16.167  1.00 41.08      B3
ATOM  2392 1H21 GLN  346      17.088  13.724  15.323  1.00  0.00      B3
ATOM  2393 2H22 GLN  346      17.026  13.063  16.919  1.00  0.00      B3
ATOM  2394 C   GLN  346      22.564  15.051  13.773  1.00 35.73      B3
ATOM  2395 O   GLN  346      22.766  16.233  14.051  1.00 38.18      B3

ATOM  2396 N   ARG  347      23.507  14.190  13.431  1.00 35.57      B3
ATOM  2397 H   ARG  347      23.248  13.289  13.157  1.00  0.00      B3
ATOM  2398 CA  ARG  347      24.907  14.538  13.396  1.00 35.95      B3
ATOM  2399 CB  ARG  347      25.760  13.236  13.222  1.00 36.20      B3
ATOM  2400 CG  ARG  347      26.198  12.549  14.540  1.00 37.41      B3
ATOM  2401 CD  ARG  347      26.986  11.246  14.373  1.00 39.70      B3
ATOM  2402 NE  ARG  347      26.072  10.167  14.028  1.00 47.18      B3
ATOM  2403 HE  ARG  347      25.416   9.893  14.701  1.00  0.00      B3
ATOM  2404 CZ  ARG  347      26.071   9.516  12.846  1.00 48.49      B3
ATOM  2405 NH1 ARG  347      26.938   9.802  11.882  1.00 50.22      B3
ATOM  2406 1H11 ARG  347      27.602  10.528  12.031  1.00  0.00      B3
ATOM  2407 1H12 ARG  347      26.905   9.313  11.011  1.00  0.00      B3
ATOM  2408 NH2 ARG  347      25.130   8.608  12.574  1.00 48.46      B3
ATOM  2409 1H21 ARG  347      24.423   8.408  13.252  1.00  0.00      B3
ATOM  2410 1H22 ARG  347      25.126   8.131  11.697  1.00  0.00      B3
ATOM  2411 C   ARG  347      25.183  15.544  12.267  1.00 35.54      B3
ATOM  2412 O   ARG  347      25.877  16.549  12.445  1.00 36.73      B3
ATOM  2413 N   ARG  348      24.611  15.353  11.096  1.00 34.74      B3
ATOM  2414 H   ARG  348      24.043  14.559  11.005  1.00  0.00      B3
ATOM  2415 CA  ARG  348      24.802  16.225   9.954  1.00 35.74      B3
ATOM  2416 CB  ARG  348      24.091  15.623   8.751  1.00 36.76      B3
ATOM  2417 CG  ARG  348      24.778  14.303   8.450  1.00 44.22      B3
ATOM  2418 CD  ARG  348      24.137  13.379   7.529  1.00 49.23      B3
ATOM  2419 NE  ARG  348      24.705  12.090   7.457  1.00 54.27      B3
ATOM  2420 HE  ARG  348      25.300  11.836   8.193  1.00  0.00      B3
ATOM  2421 CZ  ARG  348      24.557  11.226   6.430  1.00 55.75      B3
ATOM  2422 NH1 ARG  348      23.758  11.479   5.381  1.00 51.85      B3
ATOM  2423 1H11 ARG  348      23.234  12.329   5.349  1.00  0.00      B3
ATOM  2424 1H12 ARG  348      23.680  10.807   4.645  1.00  0.00      B3
ATOM  2425 NH2 ARG  348      25.252  10.083   6.467  1.00 54.51      B3
ATOM  2426 1H21 ARG  348      25.169   9.424   5.714  1.00  0.00      B3
ATOM  2427 1H22 ARG  348      25.860   9.894   7.232  1.00  0.00      B3
ATOM  2428 C   ARG  348      24.283  17.629  10.237  1.00 34.80      B3
ATOM  2429 O   ARG  348      25.078  18.564  10.219  1.00 35.16      B3
ATOM  2430 N   ALA  349      23.008  17.795  10.607  1.00 33.85      B3
ATOM  2431 H   ALA  349      22.470  16.984  10.755  1.00  0.00      B3
ATOM  2432 CA  ALA  349      22.352  19.083  10.854  1.00 32.96      B3
ATOM  2433 CB  ALA  349      20.809  18.894  11.070  1.00 33.16      B3
ATOM  2434 C   ALA  349      22.945  19.746  12.083  1.00 31.84      B3
ATOM  2435 O   ALA  349      22.981  20.969  12.210  1.00 30.69      B3
ATOM  2436 N   GLY  350      23.444  18.954  13.018  1.00 31.30      B3
ATOM  2437 H   GLY  350      23.308  17.984  12.976  1.00  0.00      B3
ATOM  2438 CA  GLY  350      24.117  19.505  14.181  1.00 31.08      B3
ATOM  2439 C   GLY  350      25.462  20.025  13.753  1.00 30.79      B3
ATOM  2440 O   GLY  350      25.974  21.010  14.280  1.00 31.48      B3
ATOM  2441 N   GLY  351      25.991  19.374  12.731  1.00 30.03      B3
ATOM  2442 H   GLY  351      25.546  18.582  12.367  1.00  0.00      B3
ATOM  2443 CA  GLY  351      27.263  19.735  12.184  1.00 29.95      B3
ATOM  2444 C   GLY  351      27.182  21.097  11.534  1.00 29.25      B3
ATOM  2445 O   GLY  351      27.937  21.974  11.919  1.00 28.71      B3
ATOM  2446 N   VAL  352      26.336  21.285  10.522  1.00 28.92      B3
```

FIGURE 5

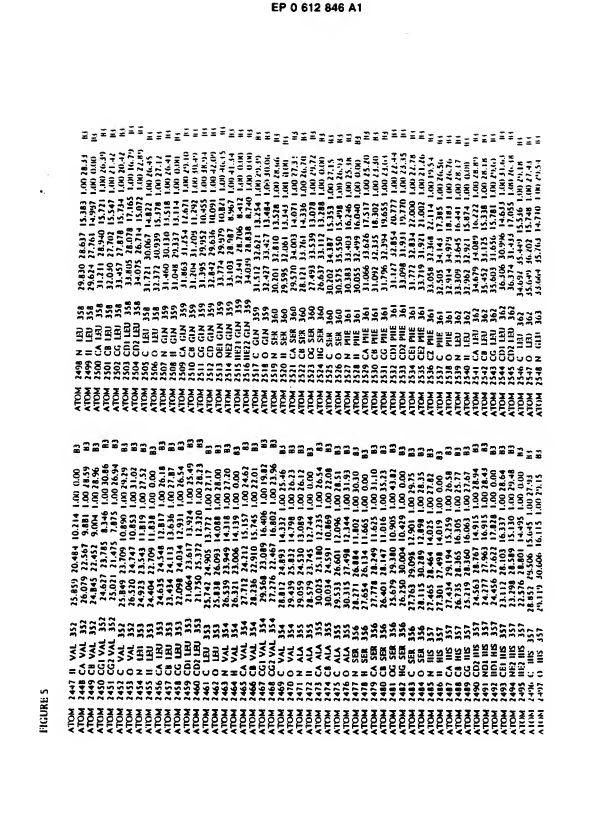

FIGURE 5

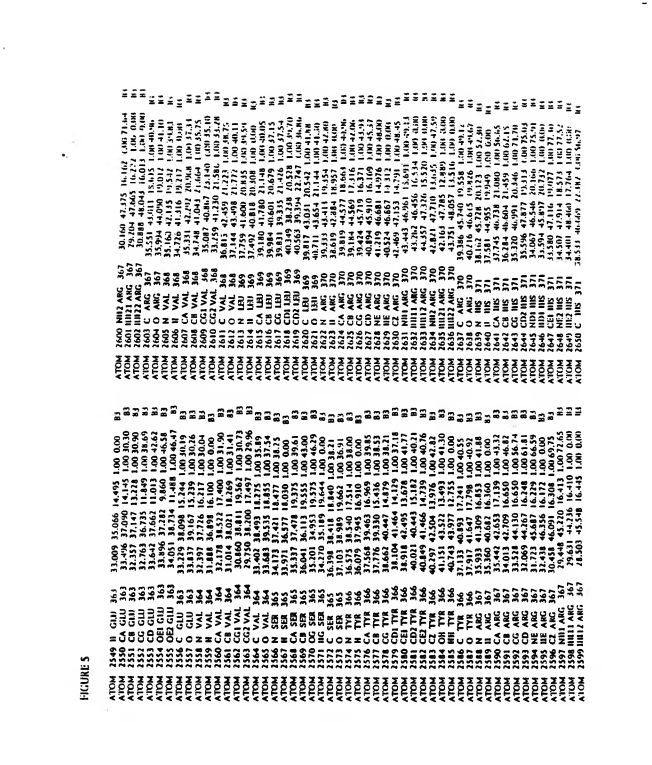

FIGURE 5

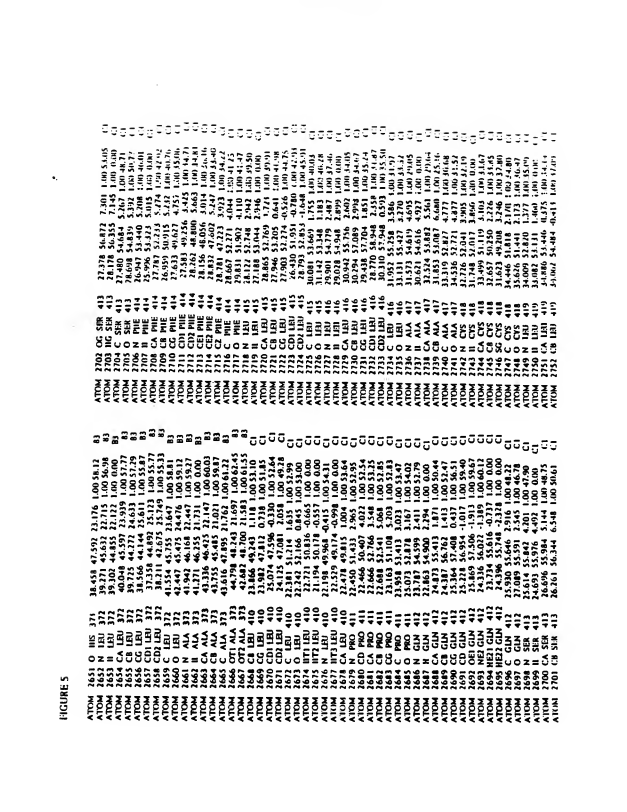

FIGURE 5

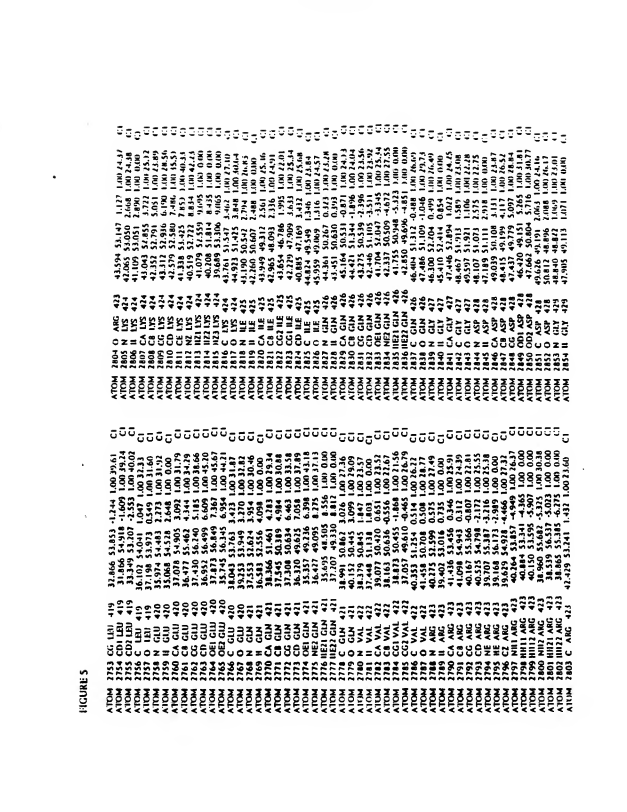

FIGURE 5


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ATOM  2855 CA GLY  429     49.289 47.964  0.029 1.00 25.44      C1
ATOM  2856 C  GLY  429     50.405 48.649 -0.716 1.00 27.39      C1
ATOM  2857 O  GLY  429     51.528 48.135 -0.741 1.00 28.51      C1
ATOM  2858 N  ALA  430     50.127 49.840 -1.271 1.00 28.26      C1
ATOM  2859 H  ALA  430     49.216 50.185 -1.172 1.00 0.00       C1
ATOM  2860 CA ALA  430     51.094 50.643 -2.015 1.00 26.04      C1
ATOM  2861 CB ALA  430     50.490 51.976 -2.407 1.00 27.93      C1
ATOM  2862 C  ALA  430     52.300 50.927 -1.133 1.00 25.19      C1
ATOM  2863 O  ALA  430     53.393 51.053 -1.655 1.00 25.43      C1
ATOM  2864 N  ALA  431     52.171 50.979  0.186 1.00 24.05      C1
ATOM  2865 H  ALA  431     51.279 50.872  0.579 1.00 0.00       C1
ATOM  2866 CA ALA  431     53.295 51.213  1.035 1.00 26.29      C1
ATOM  2867 CB ALA  431     52.874 51.522  2.458 1.00 24.14      C1
ATOM  2868 C  ALA  431     54.139 49.972  1.073 1.00 29.82      C1
ATOM  2869 O  ALA  431     55.360 50.085  0.959 1.00 31.97      C1
ATOM  2870 N  LEU  432     53.562 48.777  1.203 1.00 31.87      C1
ATOM  2871 H  LEU  432     52.585 48.726  1.279 1.00 0.00       C1
ATOM  2872 CA LEU  432     54.337 47.540  1.165 1.00 33.92      C1
ATOM  2873 CB LEU  432     53.430 46.315  1.301 1.00 37.43      C1
ATOM  2874 CG LEU  432     54.063 44.952  1.574 1.00 37.40      C1
ATOM  2875 CD1 LEU  432    54.751 44.949  2.950 1.00 38.10      C1
ATOM  2876 CD2 LEU  432    52.966 43.901  1.492 1.00 36.27      C1
ATOM  2877 C  LEU  432     55.096 47.404 -0.146 1.00 33.74      C1
ATOM  2878 O  LEU  432     56.306 47.179 -0.138 1.00 33.79      C1
ATOM  2879 N  GLN  433     54.401 47.564 -1.276 1.00 33.52      C1
ATOM  2880 H  GLN  433     53.439 47.733 -1.186 1.00 0.00       C1
ATOM  2881 CA GLN  433     55.002 47.576 -2.600 1.00 35.83      C1
ATOM  2882 CB GLN  433     53.999 47.892 -3.664 1.00 35.52      C1
ATOM  2883 CG GLN  433     52.996 46.823 -3.832 1.00 39.40      C1
ATOM  2884 CD GLN  433     52.049 47.093 -4.983 1.00 42.46      C1
ATOM  2885 OE1 GLN  433    50.924 47.576 -4.786 1.00 48.22      C1
ATOM  2886 NE2 GLN  433    52.376 46.878 -6.225 1.00 44.77      C1
ATOM  2887 HE21 GLN  433   53.271 46.540 -6.433 1.00 0.00       C1
ATOM  2888 HE22 GLN  433   51.693 47.087 -6.892 1.00 0.00       C1
ATOM  2889 C  GLN  433     56.177 48.485 -2.757 1.00 36.48      C1
ATOM  2890 O  GLN  433     57.214 48.118 -3.312 1.00 38.08      C1
ATOM  2891 N  GLU  434     56.055 49.710 -2.287 1.00 36.11      C1
ATOM  2892 H  GLU  434     55.210 49.978 -1.854 1.00 0.00       C1
ATOM  2893 CA GLU  434     57.089 50.719 -2.426 1.00 35.93      C1
ATOM  2894 CB GLU  434     56.408 52.030 -2.068 1.00 41.28      C1
ATOM  2895 CG GLU  434     57.126 53.356 -2.019 1.00 43.07      C1
ATOM  2896 CD GLU  434     57.832 53.516 -0.698 1.00 45.70      C1
ATOM  2897 OE1 GLU  434    57.190 53.538  0.367 1.00 49.33      C1
ATOM  2898 OE2 GLU  434    59.051 53.579 -0.760 1.00 45.45      C1
ATOM  2899 C  GLU  434     58.257 50.348 -1.548 1.00 34.00      C1
ATOM  2900 O  GLU  434     59.388 50.481 -1.983 1.00 32.93      C1
ATOM  2901 N  LYS  435     58.067 49.860 -0.330 1.00 34.34      C1
ATOM  2902 H  LYS  435     57.146 49.837  0.014 1.00 0.00       C1
ATOM  2903 CA LYS  435     59.151 49.358  0.511 1.00 34.56      C1
ATOM  2904 CB LYS  435     58.577 49.010  1.847 1.00 33.89      C1
ATOM  2905 CG LYS  435     58.157 50.231  2.709 1.00 36.71      C1
ATOM  2906 CD LYS  435     58.244 49.748 -4.137 1.00 40.41      C1
ATOM  2907 CE LYS  435     58.293 50.861  5.213 1.00 45.32      C1
ATOM  2908 NZ LYS  435     58.494 50.325  6.575 1.00 47.31      C1
ATOM  2909 HZ1 LYS  435    59.388 49.795  6.611 1.00 0.00       C1
ATOM  2910 HZ2 LYS  435    57.708 49.689  6.818 1.00 0.00       C1
ATOM  2911 HZ3 LYS  435    58.534 51.109  7.257 1.00 0.00       C1
ATOM  2912 C  LYS  435     59.906 48.135 -0.065 1.00 36.10      C1
ATOM  2913 O  LYS  435     61.119 48.016 -0.012 1.00 37.08      C1
ATOM  2914 N  LEU  436     59.215 47.168 -0.665 1.00 36.28      C1
ATOM  2915 H  LEU  436     58.235 47.245 -0.651 1.00 0.00       C1
ATOM  2916 CA LEU  436     59.793 45.994 -1.304 1.00 34.25      C1
ATOM  2917 CB LEU  436     58.655 45.076 -1.753 1.00 34.41      C1
ATOM  2918 CG LEU  436     57.920 44.327 -0.610 1.00 34.72      C1
ATOM  2919 CD1 LEU  436    56.764 43.518 -1.181 1.00 33.50      C1
ATOM  2920 CD2 LEU  436    58.880 43.375  0.117 1.00 36.89      C1
ATOM  2921 C  LEU  436     60.669 46.383 -2.467 1.00 33.31      C1
ATOM  2922 O  LEU  436     61.756 45.825 -2.647 1.00 33.94      C1
ATOM  2923 N  CYS  437     60.220 47.374 -3.222 1.00 32.34      C1
ATOM  2924 H  CYS  437     59.290 47.661 -3.097 1.00 0.00       C1
ATOM  2925 CA CYS  437     60.978 47.949 -4.301 1.00 32.01      C1
ATOM  2926 C  CYS  437     62.214 48.704 -3.857 1.00 34.70      C1
ATOM  2927 O  CYS  437     63.148 48.599 -4.412 1.00 36.26      C1
ATOM  2928 CB CYS  437     60.094 48.840 -5.008 1.00 30.77      C1
ATOM  2929 SG CYS  437     61.003 49.666 -6.319 1.00 36.22      C1
ATOM  2930 N  ALA  438     62.016 49.463 -2.785 1.00 36.35      C1
ATOM  2931 H  ALA  438     61.108 49.547 -2.431 1.00 0.00       C1
ATOM  2932 CA ALA  438     63.060 50.226 -2.170 1.00 35.83      C1
ATOM  2933 CB ALA  438     62.553 51.107 -1.153 1.00 36.58      C1
ATOM  2934 C  ALA  438     64.065 49.294 -1.527 1.00 37.01      C1
ATOM  2935 O  ALA  438     65.172 49.168 -2.092 1.00 39.19      C1
ATOM  2936 N  THR  439     63.808 48.591 -0.422 1.00 36.59      C1
ATOM  2937 H  THR  439     62.947 48.723  0.014 1.00 0.00       C1
ATOM  2938 CA THR  439     64.742 47.669  0.223 1.00 35.70      C1
ATOM  2939 CB THR  439     64.073 47.042  1.400 1.00 35.34      C1
ATOM  2940 OG1 THR  439    63.323 48.048  2.040 1.00 38.83      C1
ATOM  2941 HG1 THR  439    62.419 47.999  1.706 1.00 0.00       C1
ATOM  2942 CG2 THR  439    65.019 46.479  2.369 1.00 36.50      C1
ATOM  2943 C  THR  439     65.331 46.517 -0.590 1.00 46.10      C1
ATOM  2944 O  THR  439     66.448 46.093 -0.312 1.00 36.54      C1
ATOM  2945 N  TYR  440     64.603 45.917 -1.548 1.00 46.02      C1
ATOM  2946 H  TYR  440     63.751 46.319 -1.822 1.00 0.00       C1
ATOM  2947 CA TYR  440     65.057 44.691 -2.198 1.00 34.78      C1
ATOM  2948 CB TYR  440     64.175 44.480 -3.878 1.00 43.09      C1
ATOM  2949 CG TYR  440     64.016 43.240 -0.397 1.00 34.14      C1
ATOM  2950 CD1 TYR  440    62.773 43.230  0.169 1.00 35.16      C1
ATOM  2951 CE1 TYR  440    62.625 44.017  1.532 1.00 36.66      C1
ATOM  2952 CD2 TYR  440    65.126 45.064  0.385 1.00 37.83      C1
ATOM  2953 CE2 TYR  440    64.992 42.881  1.752 1.00 39.02      C1
ATOM  2954 CZ  TYR  440    63.743 42.864  2.317 1.00 37.34      C1
ATOM  2955 OH  TYR  440    63.637 42.649  3.678 1.00 47.56      C1
ATOM  2956 HH  TYR  440    64.498 47.343  3.988 1.00 0.00       C1
```

FIGURE 5

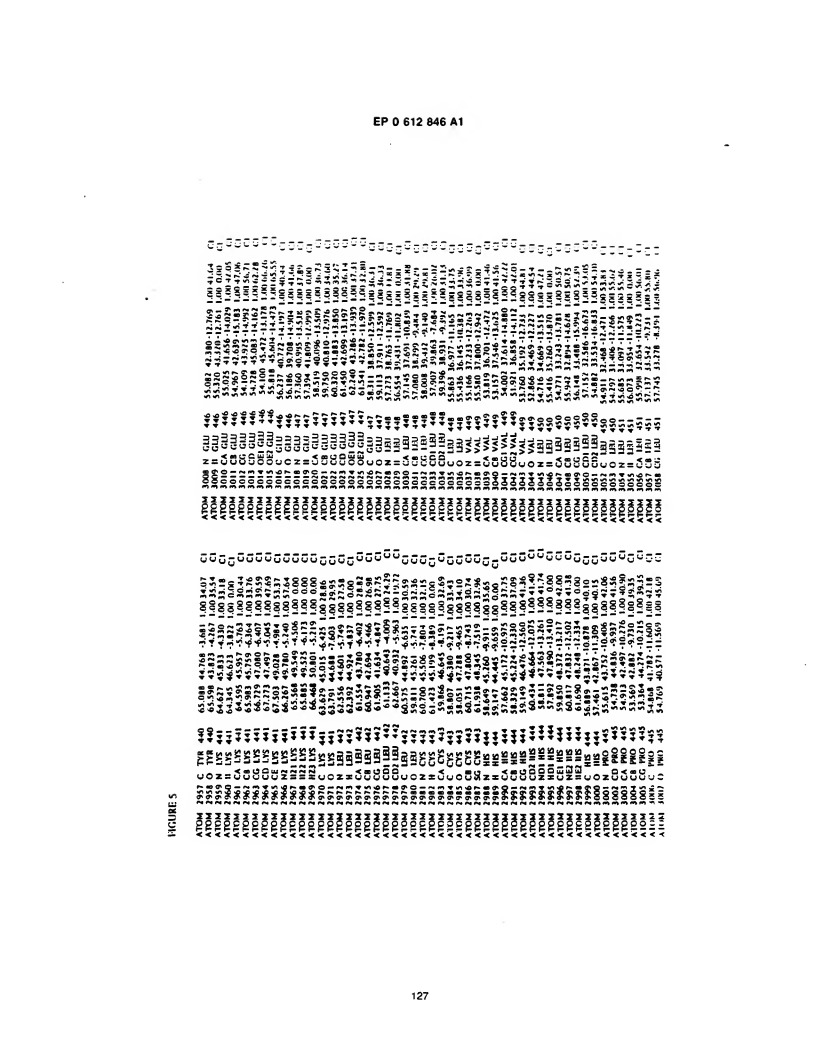


FIGURE 5

FIGURE 5

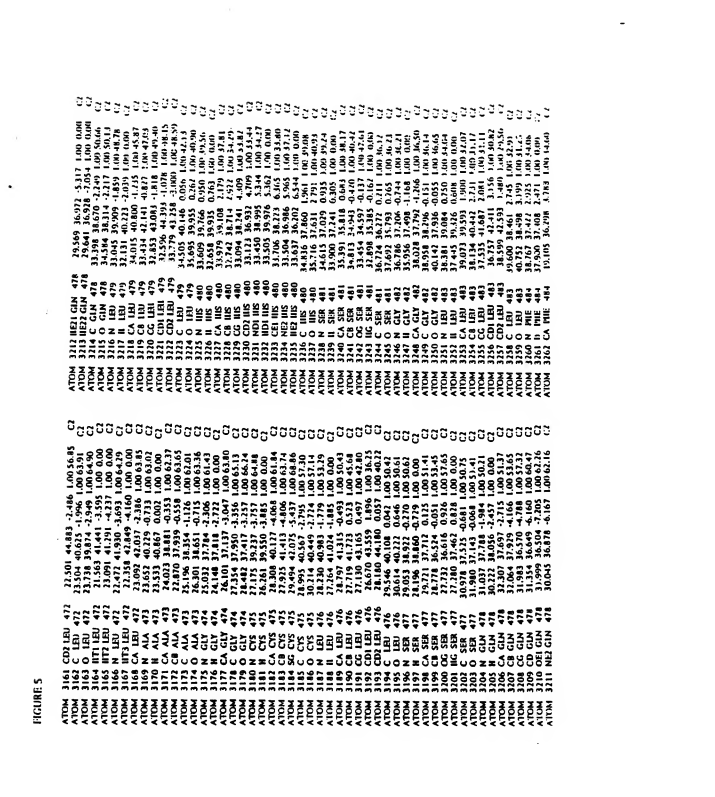


FIGURE 5

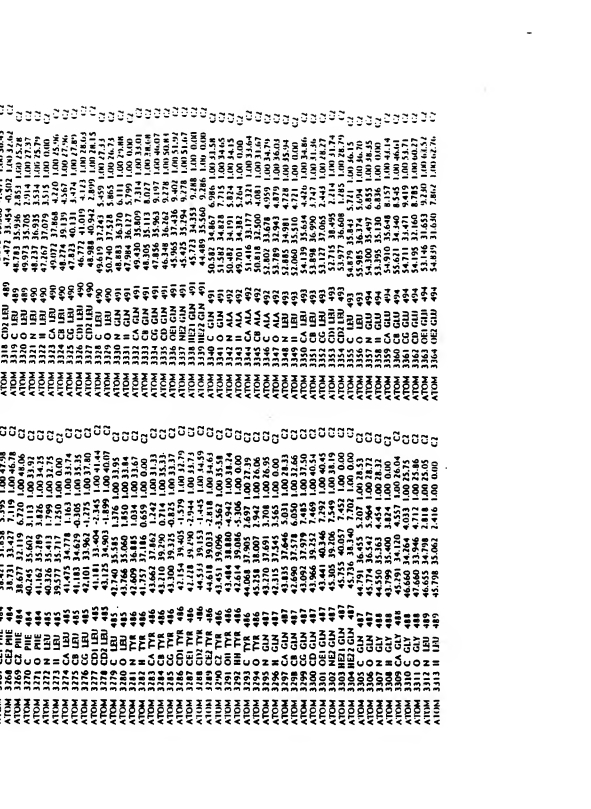


FIGURE 5

```
ATOM  3365 C  GLU  494      55.865  36.825   8.343  1.00 44.32      C2
ATOM  3366 O  GLU  494      57.055  36.678   8.610  1.00 46.91      C2
ATOM  3367 N  GLY  495      55.358  38.046   8.114  1.00 44.32      C2
ATOM  3368 H  GLY  495      54.450  38.112   7.753  1.00  0.00      C2
ATOM  3369 CA GLY  495      56.104  39.272   8.368  1.00 42.36      C2
ATOM  3370 C  GLY  495      57.015  39.695   7.238  1.00 42.33      C2
ATOM  3371 O  GLY  495      57.397  40.856   7.220  1.00 42.42      C2
ATOM  3372 N  ILE  496      57.310  38.802   6.279  1.00 41.04      C2
ATOM  3373 H  ILE  496      56.927  37.906   6.374  1.00  0.00      C2
ATOM  3374 CA ILE  496      58.259  38.993   5.192  1.00 41.15      C2
ATOM  3375 CB ILE  496      57.929  40.216   4.253  1.00 38.60      C2
ATOM  3376 CG2 ILE  496      59.077  40.437   3.248  1.00 37.62      C2
ATOM  3377 CG1 ILE  496      56.662  39.964   3.480  1.00 36.39      C2
ATOM  3378 CD ILE  496      56.314  41.071   2.470  1.00 35.27      C2
ATOM  3379 C  ILE  496      59.672  39.203   5.749  1.00 42.91      C2
ATOM  3380 O  ILE  496      60.541  38.396   5.448  1.00 44.22      C2
ATOM  3381 N  SER  497      59.998  40.228   6.533  1.00 44.31      C2
ATOM  3382 H  SER  497      59.297  40.852   6.827  1.00  0.00      C2
ATOM  3383 CA SER  497      61.346  40.501   6.992  1.00 44.86      C2
ATOM  3384 CB SER  497      62.204  41.254   5.938  1.00 44.13      C2
ATOM  3385 OG SER  497      62.181  42.673   6.033  1.00 40.74      C2
ATOM  3386 HG SER  497      62.531  42.964   5.170  1.00  0.00      C2
ATOM  3387 C  SER  497      61.164  41.413   8.185  1.00 45.85      C2
ATOM  3388 O  SER  497      60.132  42.110   8.288  1.00 47.55      C2
ATOM  3389 N  PRO  498      62.164  41.490   9.071  1.00 44.96      C2
ATOM  3390 CD PRO  498      63.338  40.621   9.126  1.00 42.13      C2
ATOM  3391 CA PRO  498      62.086  42.337  10.250  1.00 44.88      C2
ATOM  3392 HA PRO  498      63.431  42.038  10.885  1.00 45.13      C2
ATOM  3393 CG PRO  498      63.629  40.541  10.603  1.00 42.00      C2
ATOM  3394 C  PRO  498      61.760  43.799   9.983  1.00 45.22      C2
ATOM  3395 O  PRO  498      61.215  44.446  10.869  1.00 45.24      C2
ATOM  3396 N  GLU  499      62.017  44.314   8.777  1.00 46.15      C2
ATOM  3397 H  GLU  499      62.362  43.716   8.081  1.00  0.00      C2
ATOM  3398 CA GLU  499      61.731  45.699   8.391  1.00 48.06      C2
ATOM  3399 CB GLU  499      62.498  46.193   7.155  1.00 52.19      C2
ATOM  3400 CG GLU  499      64.001  46.187   7.100  1.00 57.51      C2
ATOM  3401 CD GLU  499      64.544  44.737   7.076  1.00 60.61      C2
ATOM  3402 OE1 GLU  499      64.755  44.231   8.162  1.00 62.96      C2
ATOM  3403 OE2 GLU  499      64.739  44.234   5.984  1.00 62.79      C2
ATOM  3404 C  GLU  499      60.269  45.896   7.981  1.00 46.94      C2
ATOM  3405 O  GLU  499      59.600  46.895   8.277  1.00 48.15      C2
ATOM  3406 N  LEU  500      59.806  44.934   7.193  1.00 44.38      C2
ATOM  3407 H  LEU  500      60.351  44.137   7.027  1.00  0.00      C2
ATOM  3408 CA LEU  500      58.491  44.997   6.651  1.00 41.08      C2
ATOM  3409 CB LEU  500      58.519  44.197   5.445  1.00 41.37      C2
ATOM  3410 CG LEU  500      59.303  44.862   4.351  1.00 42.70      C2
ATOM  3411 CD1 LEU  500      59.776  43.828   3.351  1.00 43.98      C2
ATOM  3412 CD2 LEU  500      58.427  45.874   3.671  1.00 45.04      C2
ATOM  3413 C  LEU  500      57.455  44.521   7.628  1.00 40.59      C2
ATOM  3414 O  LEU  500      56.274  44.835   7.463  1.00 40.61      C2
ATOM  3415 N  GLY  501      57.866  43.835   8.685  1.00 39.37      C2

ATOM  3416 H  GLY  501      58.808  43.579   8.730  1.00  0.00      C2
ATOM  3417 CA GLY  501      56.974  43.386   9.734  1.00 39.59      C2
ATOM  3418 C  GLY  501      55.816  44.324  10.091  1.00 39.66      C2
ATOM  3419 O  GLY  501      54.661  44.034   9.777  1.00 40.66      C2
ATOM  3420 N  PRO  502      55.986  45.462  10.742  1.00 39.90      C2
ATOM  3421 CD PRO  502      57.237  45.908  11.335  1.00 41.18      C2
ATOM  3422 CA PRO  502      54.912  46.387  11.045  1.00 38.67      C2
ATOM  3423 CB PRO  502      55.594  47.494  11.791  1.00 39.23      C2
ATOM  3424 CG PRO  502      56.989  47.405  11.221  1.00 41.36      C2
ATOM  3425 C  PRO  502      54.158  46.849   9.817  1.00 37.54      C2
ATOM  3426 O  PRO  502      52.966  47.139   9.961  1.00 38.36      C2
ATOM  3427 N  THR  503      54.728  46.887   8.609  1.00 35.11      C2
ATOM  3428 H  THR  503      55.663  46.638   8.449  1.00  0.00      C2
ATOM  3429 CA THR  503      53.940  47.283   7.462  1.00 35.09      C2
ATOM  3430 CB THR  503      54.832  47.376   6.245  1.00 34.48      C2
ATOM  3431 OG1 THR  503      56.025  48.018   6.668  1.00 38.23      C2
ATOM  3432 HG1 THR  503      55.857  48.946   6.845  1.00 00.00      C2
ATOM  3433 CG2 THR  503      54.197  48.162   5.126  1.00 35.56      C2
ATOM  3434 C  THR  503      52.826  46.252   7.215  1.00 35.37      C2
ATOM  3435 O  THR  503      51.671  46.552   6.915  1.00 37.13      C2
ATOM  3436 N  LEU  504      53.218  44.996   7.380  1.00 34.02      C2
ATOM  3437 H  LEU  504      54.146  44.799   7.647  1.00  0.00      C2
ATOM  3438 CA LEU  504      52.301  43.912   7.173  1.00 35.00      C2
ATOM  3439 CB LEU  504      53.127  42.650   7.002  1.00 34.78      C2
ATOM  3440 CG LEU  504      53.464  42.256   5.601  1.00 34.07      C2
ATOM  3441 CD1 LEU  504      54.163  40.927   5.667  1.00 37.97      C2
ATOM  3442 CD2 LEU  504      52.254  41.865   4.809  1.00 37.34      C2
ATOM  3443 C  LEU  504      51.324  43.821   8.328  1.00 29.54      C2
ATOM  3444 O  LEU  504      50.141  43.562   8.078  1.00 30.40      C2
ATOM  3445 N  ASP  505      51.736  44.106   9.551  1.00 26.09      C2
ATOM  3446 H  ASP  505      52.689  44.269   9.699  1.00  0.00      C2
ATOM  3447 CA ASP  505      50.798  44.084  10.643  1.00 27.98      C2
ATOM  3448 CB ASP  505      51.446  44.345  11.976  1.00 29.86      C2
ATOM  3449 CG ASP  505      52.500  43.312  12.239  1.00 34.64      C2
ATOM  3450 OD1 ASP  505      52.663  42.298  11.534  1.00 41.04      C2
ATOM  3451 OD2 ASP  505      53.179  43.542  13.224  1.00 37.40      C2
ATOM  3452 C  ASP  505      49.661  45.060  10.368  1.00 28.61      C2
ATOM  3453 O  ASP  505      48.566  44.739  11.039  1.00 30.30      C2
ATOM  3454 N  THR  506      49.894  46.242  10.002  1.00 28.29      C2
ATOM  3455 H  THR  506      50.823  46.493   9.804  1.00  0.00      C2
ATOM  3456 CA THR  506      48.860  47.225   9.731  1.00 25.74      C2
ATOM  3457 CB THR  506      49.497  48.556   9.316  1.00 26.14      C2
ATOM  3458 OG1 THR  506      49.944  49.099  10.548  1.00 31.63      C2
ATOM  3459 HG1 THR  506      49.243  49.072  11.246  1.00  0.00      C2
ATOM  3460 CG2 THR  506      48.594  49.517   8.619  1.00 24.46      C2
ATOM  3461 C  THR  506      48.022  46.735   8.615  1.00 24.00      C2
ATOM  3462 O  THR  506      46.817  46.864   8.719  1.00 25.85      C2
ATOM  3463 N  LEU  507      48.554  46.196   7.525  1.00 23.51      C2
ATOM  3464 H  LEU  507      49.527  46.073   7.453  1.00  0.00      C2
ATOM  3465 CA LEU  507      47.682  45.770   6.434  1.00 21.85      C2
ATOM  3466 CB LEU  507      48.574  45.408   5.196  1.00 23.31      C2
```

FIGURE 5

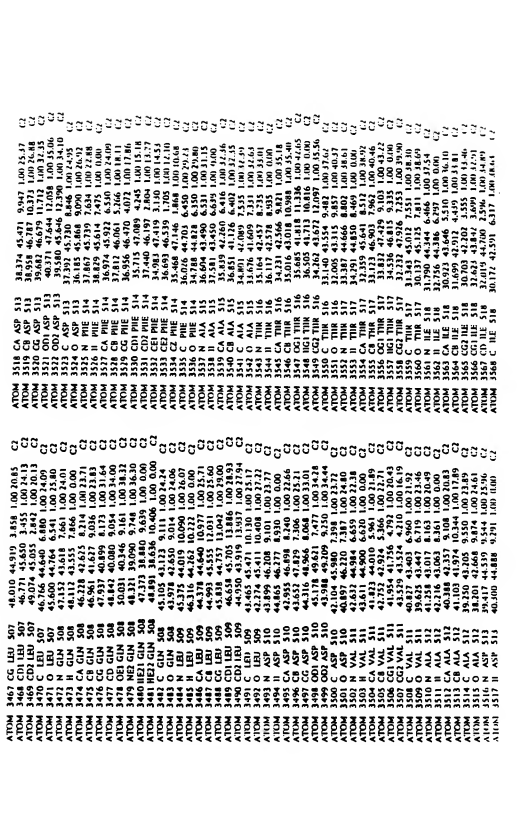
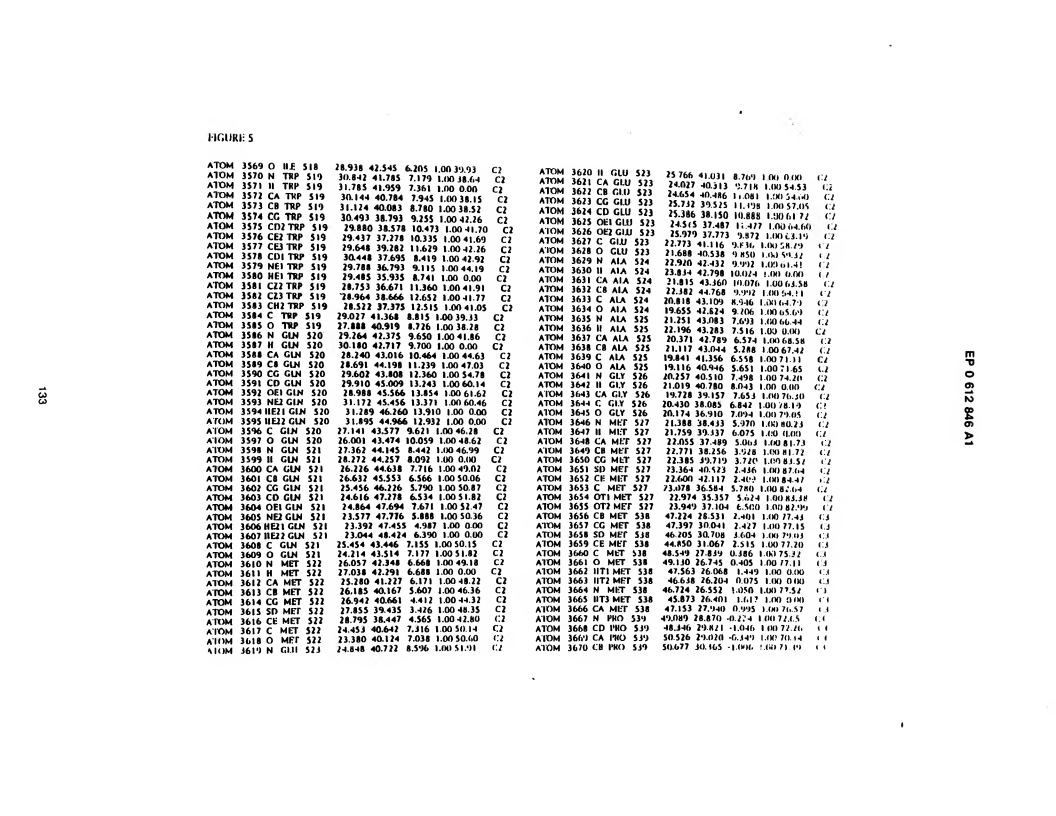


FIGURE 5



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ATOM  3569 O   ILE S18   28.938  42.545   6.205  1.00 39.93      C2
ATOM  3570 N   TRP S19   30.842  41.785   7.179  1.00 38.64      C2
ATOM  3571 H   TRP S19   31.785  41.959   7.361  1.00  0.00      C2
ATOM  3572 CA  TRP S19   30.144  40.784   7.945  1.00 38.15      C2
ATOM  3573 CB  TRP S19   31.124  40.083   8.780  1.00 38.52      C2
ATOM  3574 CG  TRP S19   30.493  38.793   9.255  1.00 42.26      C2
ATOM  3575 CD2 TRP S19   29.880  38.578  10.473  1.00 41.70      C2
ATOM  3576 CE2 TRP S19   29.437  37.278  10.335  1.00 41.69      C2
ATOM  3577 CE3 TRP S19   29.648  39.282  11.629  1.00 42.26      C2
ATOM  3578 CD1 TRP S19   30.448  37.695   8.419  1.00 42.92      C2
ATOM  3579 NE1 TRP S19   29.788  36.793   9.115  1.00 44.19      C2
ATOM  3580 HE1 TRP S19   29.485  35.935   8.741  1.00  0.00      C2
ATOM  3581 CZ2 TRP S19   28.753  36.671  11.360  1.00 41.91      C2
ATOM  3582 CZ3 TRP S19   28.964  38.666  12.652  1.00 41.77      C2
ATOM  3583 CH2 TRP S19   28.522  37.375  12.515  1.00 41.05      C2
ATOM  3584 C   TRP S19   29.027  41.368   8.815  1.00 39.33      C2
ATOM  3585 O   TRP S19   27.888  40.919   8.726  1.00 38.78      C2
ATOM  3586 N   GLN S20   29.264  42.375   9.650  1.00 41.86      C2
ATOM  3587 H   GLN S20   30.180  42.717   9.700  1.00  0.00      C2
ATOM  3588 CA  GLN S20   28.240  43.016  10.464  1.00 44.63      C2
ATOM  3589 CB  GLN S20   28.691  44.198  11.239  1.00 47.03      C2
ATOM  3590 CG  GLN S20   29.602  43.808  12.360  1.00 54.78      C2
ATOM  3591 CD  GLN S20   29.910  45.009  13.243  1.00 60.14      C2
ATOM  3592 OE1 GLN S20   28.988  45.566  13.854  1.00 61.62      C2
ATOM  3593 NE2 GLN S20   31.172  45.456  13.371  1.00 60.46      C2
ATOM  3594 HE21 GLN S20   31.289  46.260  13.910  1.00  0.00      C2
ATOM  3595 HE22 GLN S20   31.895  44.966  12.932  1.00  0.00      C2
ATOM  3596 C   GLN S20   27.141  43.571   9.621  1.00 46.28      C2
ATOM  3597 O   GLN S20   26.001  43.474  10.059  1.00 48.62      C2
ATOM  3598 N   GLN S21   27.362  44.145   8.442  1.00 46.99      C2
ATOM  3599 H   GLN S21   28.272  44.465   8.093  1.00  0.00      C2
ATOM  3600 CA  GLN S21   26.226  44.638   7.716  1.00 49.02      C2
ATOM  3601 CB  GLN S21   26.632  45.553   6.566  1.00 50.06      C2
ATOM  3602 CG  GLN S21   25.456  46.025   5.790  1.00 50.87      C2
ATOM  3603 CD  GLN S21   24.616  47.278   6.534  1.00 51.82      C2
ATOM  3604 OE1 GLN S21   24.864  47.694   7.671  1.00 52.47      C2
ATOM  3605 NE2 GLN S21   23.577  47.776   5.888  1.00 50.36      C2
ATOM  3606 HE21 GLN S21   23.392  47.455   4.987  1.00  0.00      C2
ATOM  3607 HE22 GLN S21   23.044  48.424   6.390  1.00  0.00      C2
ATOM  3608 C   GLN S21   25.454  43.465   7.155  1.00 50.15      C2
ATOM  3609 O   GLN S21   24.214  43.514   7.177  1.00 51.82      C2
ATOM  3610 N   MET S22   26.057  42.348   6.668  1.00 49.18      C2
ATOM  3611 H   MET S22   27.038  42.291   6.688  1.00  0.00      C2
ATOM  3612 CA  MET S22   25.280  41.227   6.171  1.00 48.22      C2
ATOM  3613 CB  MET S22   26.185  40.167   5.607  1.00 46.36      C2
ATOM  3614 CG  MET S22   26.942  40.661   4.412  1.00 44.32      C2
ATOM  3615 SD  MET S22   27.855  39.435   3.426  1.00 48.35      C2
ATOM  3616 CE  MET S22   28.795  38.647   4.565  1.00 42.80      C2
ATOM  3617 C   MET S22   24.453  40.642   7.316  1.00 50.14      C2
ATOM  3618 O   MET S22   23.380  40.124   7.038  1.00 50.60      C2
ATOM  3619 N   GLU S23   24.848  40.722   8.596  1.00 51.91      C2
ATOM  3620 H   GLU S23   25.766  41.031   8.769  1.00  0.00      C2
ATOM  3621 CA  GLU S23   24.027  40.313   9.718  1.00 54.53      C2
ATOM  3622 CB  GLU S23   24.654  40.486  11.081  1.00 54.49      C2
ATOM  3623 CG  GLU S23   25.732  39.525  11.498  1.00 57.05      C2
ATOM  3624 CD  GLU S23   25.386  38.150  10.888  1.00 61.72      C2
ATOM  3625 OE1 GLU S23   24.515  37.487  11.477  1.00 64.66      C2
ATOM  3626 OE2 GLU S23   25.979  37.773   9.872  1.00 63.19      C2
ATOM  3627 C   GLU S23   22.723  41.116   9.836  1.00 58.29      C2
ATOM  3628 O   GLU S23   21.688  40.538   9.850  1.00 59.32      C2
ATOM  3629 N   ALA S24   22.920  42.432   9.992  1.00 61.41      C2
ATOM  3630 H   ALA S24   23.834  42.798  10.024  1.00  0.00      C2
ATOM  3631 CA  ALA S24   21.815  43.360  10.076  1.00 63.58      C2
ATOM  3632 CB  ALA S24   22.382  44.768   9.992  1.00 64.11      C2
ATOM  3633 C   ALA S24   20.818  43.109   8.946  1.00 64.79      C2
ATOM  3634 O   ALA S24   19.655  42.824   9.206  1.00 65.69      C2
ATOM  3635 N   ALA S25   21.251  43.083   7.693  1.00 66.44      C2
ATOM  3636 H   ALA S25   22.196  43.283   7.516  1.00  0.00      C2
ATOM  3637 CA  ALA S25   20.371  42.789   6.574  1.00 68.58      C2
ATOM  3638 CB  ALA S25   21.117  43.044   5.288  1.00 67.42      C2
ATOM  3639 C   ALA S25   19.841  41.356   6.558  1.00 71.13      C2
ATOM  3640 O   ALA S25   19.116  40.946   5.651  1.00 71.65      C2
ATOM  3641 N   GLY S26   20.197  40.510   7.498  1.00 74.20      C2
ATOM  3642 H   GLY S26   21.019  40.780   8.043  1.00  0.00      C2
ATOM  3643 CA  GLY S26   19.728  39.157   7.653  1.00 76.30      C2
ATOM  3644 C   GLY S26   20.678  38.085   6.842  1.00 78.14      C2
ATOM  3645 O   GLY S26   20.174  36.910   7.094  1.00 79.05      C2
ATOM  3646 N   MET S27   21.388  38.433   5.770  1.00 80.23      C2
ATOM  3647 H   MET S27   21.758  39.337   6.075  1.00  0.00      C2
ATOM  3648 CA  MET S27   22.055  37.489   5.063  1.00 81.73      C2
ATOM  3649 CB  MET S27   22.771  38.256   3.928  1.00 81.72      C2
ATOM  3650 CG  MET S27   23.785  39.319   3.720  1.00 83.52      C2
ATOM  3651 SD  MET S27   23.364  40.423   2.436  1.00 87.64      C2
ATOM  3652 CE  MET S27   22.600  42.117   2.402  1.00 84.47      C2
ATOM  3653 C   MET S27   23.078  36.584   5.780  1.00 82.26      C2
ATOM  3654 OT1 MET S27   22.974  35.357   5.624  1.00 83.58      C2
ATOM  3655 OT2 MET S27   23.949  37.104   6.560  1.00 82.99      C2
ATOM  3656 CB  MET S38   47.224  28.531   2.401  1.00 77.43      C3
ATOM  3657 CG  MET S38   47.397  30.041   2.427  1.00 77.15      C3
ATOM  3658 SD  MET S38   46.205  30.708   3.604  1.00 79.03      C3
ATOM  3659 CE  MET S38   44.850  31.067   2.515  1.00 77.36      C3
ATOM  3660 C   MET S38   48.549  27.839   0.386  1.00 75.32      C3
ATOM  3661 O   MET S38   49.130  26.745   0.405  1.00 77.11      C3
ATOM  3662 1IT1 MET S38   47.563  26.068   1.449  1.00  0.00      C3
ATOM  3663 1IT2 MET S38   46.638  26.204   0.075  1.00  0.00      C3
ATOM  3664 N   MET S38   46.724  26.552   1.050  1.00 77.52      C3
ATOM  3665 1IT3 MET S38   45.873  26.401   1.617  1.00  0.00      C3
ATOM  3666 CA  MET S38   47.153  27.940   0.995  1.00 76.57      C3
ATOM  3667 N   PRO S39   49.089  28.870  -0.224  1.00 72.65      C4
ATOM  3668 CD  PRO S39   48.346  29.821  -1.046  1.00 72.76      C4
ATOM  3669 CA  PRO S39   50.526  29.028  -0.549  1.00 70.14      C4
ATOM  3670 CB  PRO S39   50.677  30.465  -1.006  1.00 71.09      C4
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FIGURE 5

```
ATOM  3671 CG  PRO  539      49.437  30.503  -1.837  1.00 71.52      C3
ATOM  3672 C   PRO  539      51.250  28.931   0.991  1.00 67.83      C3
ATOM  3673 O   PRO  539      50.666  29.294   2.029  1.00 68.05      C3
ATOM  3674 N   ALA  540      52.484  28.417   0.961  1.00 64.48      C3
ATOM  3675 H   ALA  540      52.858  28.098   0.111  1.00  0.00      C3
ATOM  3676 CA  ALA  540      53.389  28.498   2.112  1.00 61.03      C3
ATOM  3677 CB  ALA  540      54.004  27.200   2.619  1.00 63.57      C3
ATOM  3678 C   ALA  540      54.559  29.212   1.496  1.00 58.74      C3
ATOM  3679 O   ALA  540      54.835  29.036   0.301  1.00 58.30      C3
ATOM  3680 N   PHE  541      55.256  30.008   2.292  1.00 55.25      C3
ATOM  3681 H   PHE  541      55.093  30.068   3.257  1.00  0.00      C3
ATOM  3682 CA  PHE  541      56.299  30.814   1.702  1.00 51.38      C3
ATOM  3683 CB  PHE  541      55.964  32.306   1.942  1.00 48.80      C3
ATOM  3684 CG  PHE  541      54.789  32.703   1.058  1.00 45.77      C3
ATOM  3685 CD1 PHE  541      54.992  32.939  -0.279  1.00 44.20      C3
ATOM  3686 CD2 PHE  541      53.507  32.747   1.582  1.00 44.76      C3
ATOM  3687 CE1 PHE  541      53.901  33.307  -1.074  1.00 43.98      C3
ATOM  3688 CE2 PHE  541      52.418  33.018   0.769  1.00 42.86      C3
ATOM  3689 CZ  PHE  541      52.625  33.247  -0.563  1.00 42.52      C3
ATOM  3690 C   PHE  541      57.586  30.364   2.333  1.00 49.80      C3
ATOM  3691 O   PHE  541      58.002  30.807   3.395  1.00 49.55      C3
ATOM  3692 N   ALA  542      58.172  29.464   1.562  1.00 48.71      C3
ATOM  3693 H   ALA  542      57.825  29.298   0.656  1.00  0.00      C3
ATOM  3694 CA  ALA  542      59.326  28.711   1.968  1.00 45.37      C3
ATOM  3695 CB  ALA  542      59.700  27.749   0.898  1.00 45.21      C3
ATOM  3696 C   ALA  542      60.510  29.567   2.266  1.00 44.87      C3
ATOM  3697 O   ALA  542      61.001  29.504   3.374  1.00 46.49      C3
ATOM  3698 N   SER  543      61.013  30.408   1.395  1.00 42.63      C3
ATOM  3699 H   SER  543      60.477  30.685   0.630  1.00  0.00      C3
ATOM  3700 CA  SER  543      62.253  31.108   1.708  1.00 40.31      C3
ATOM  3701 CB  SER  543      63.170  30.861   0.587  1.00 37.74      C3
ATOM  3702 OG  SER  543      62.391  31.181  -0.554  1.00 35.74      C3
ATOM  3703 HG  SER  543      61.824  30.423  -0.751  1.00  0.00      C3
ATOM  3704 C   SER  543      62.087  32.613   1.896  1.00 40.48      C3
ATOM  3705 O   SER  543      61.016  33.115   1.536  1.00 42.63      C3
ATOM  3706 N   ALA  544      63.120  33.383   2.310  1.00 38.84      C3
ATOM  3707 H   ALA  544      63.929  32.951   2.650  1.00  0.00      C3
ATOM  3708 CA  ALA  544      63.035  34.836   2.345  1.00 37.31      C3
ATOM  3709 CB  ALA  544      64.340  35.450   2.808  1.00 35.74      C3
ATOM  3710 C   ALA  544      62.723  35.372   0.947  1.00 37.06      C3
ATOM  3711 O   ALA  544      61.829  36.220   0.820  1.00 38.23      C3
ATOM  3712 N   PHE  545      63.357  34.881  -0.130  1.00 35.72      C3
ATOM  3713 H   PHE  545      64.131  34.298  -0.010  1.00  0.00      C3
ATOM  3714 CA  PHE  545      62.992  35.268  -1.484  1.00 33.66      C3
ATOM  3715 CB  PHE  545      63.738  34.534  -2.593  1.00 29.71      C3
ATOM  3716 CG  PHE  545      63.140  34.742  -3.990  1.00 29.62      C3
ATOM  3717 CD1 PHE  545      62.317  33.788  -4.557  1.00 29.80      C3
ATOM  3718 CD2 PHE  545      63.371  35.915  -4.689  1.00 31.90      C3
ATOM  3719 CE1 PHE  545      61.723  33.984  -5.795  1.00 28.61      C3
ATOM  3720 CE2 PHE  545      62.777  36.113  -5.928  1.00 31.00      C3
ATOM  3721 CZ  PHE  545      61.955  35.150  -6.480  1.00 31.01      C3
ATOM  3722 C   PHE  545      61.543  34.900  -1.667  1.00 34.81      C3
ATOM  3723 O   PHE  545      60.901  35.660  -2.389  1.00 38.88      C3
ATOM  3724 N   GLN  546      60.912  33.847  -1.135  1.00 34.77      C3
ATOM  3725 H   GLN  546      61.396  33.223  -0.558  1.00  0.00      C3
ATOM  3726 CA  GLN  546      59.490  33.647  -1.433  1.00 34.72      C3
ATOM  3727 CB  GLN  546      59.145  32.232  -1.140  1.00 34.85      C3
ATOM  3728 CG  GLN  546      59.582  31.385  -2.444  1.00 42.45      C3
ATOM  3729 CD  GLN  546      59.374  30.085  -2.473  1.00 46.95      C3
ATOM  3730 OE1 GLN  546      59.287  29.472  -1.399  1.00 48.90      C3
ATOM  3731 NE2 GLN  546      59.339  29.442  -3.644  1.00 47.20      C3
ATOM  3732 HE21 GLN  546      59.476  29.946  -4.472  1.00  0.00      C3
ATOM  3733 HE22 GLN  546      59.154  28.481  -3.609  1.00  0.00      C3
ATOM  3734 C   GLN  546      58.504  34.541  -0.729  1.00 31.54      C3
ATOM  3735 O   GLN  546      57.429  34.850  -1.239  1.00 29.88      C3
ATOM  3736 N   ARG  547      58.907  34.929   0.465  1.00 31.09      C3
ATOM  3737 H   ARG  547      59.750  34.566   0.811  1.00  0.00      C3
ATOM  3738 CA  ARG  547      58.160  35.830   1.282  1.00 31.45      C3
ATOM  3739 CB  ARG  547      58.813  35.874   2.601  1.00 31.74      C3
ATOM  3740 CG  ARG  547      57.906  35.224   3.623  1.00 37.02      C3
ATOM  3741 CD  ARG  547      58.344  33.858   4.076  1.00 40.56      C3
ATOM  3742 NE  ARG  547      59.743  34.058   4.345  1.00 47.90      C3
ATOM  3743 HE  ARG  547      60.349  33.924   3.620  1.00  0.00      C3
ATOM  3744 CZ  ARG  547      60.190  34.394   5.543  1.00 49.48      C3
ATOM  3745 NH1 ARG  547      59.361  34.522   6.593  1.00 51.97      C3
ATOM  3746 HH11 ARG  547      58.380  34.356   6.488  1.00  0.00      C3
ATOM  3747 HH12 ARG  547      59.731  34.763   7.491  1.00  0.00      C3
ATOM  3748 NH2 ARG  547      61.464  34.775   5.616  1.00 48.53      C3
ATOM  3749 HH21 ARG  547      62.025  34.801   4.788  1.00  0.00      C3
ATOM  3750 HH22 ARG  547      61.854  35.034   6.501  1.00  0.00      C3
ATOM  3751 C   ARG  547      56.167  37.181   0.590  1.00 32.66      C3
ATOM  3752 O   ARG  547      57.084  37.694   0.317  1.00 34.25      C3
ATOM  3753 N   ARG  548      55.348  37.717   0.205  1.00 31.24      C3
ATOM  3754 H   ARG  548      60.148  37.203   0.444  1.00  0.00      C3
ATOM  3755 CA  ARG  548      59.529  38.980  -0.555  1.00 30.01      C3
ATOM  3756 CB  ARG  548      60.995  39.213  -0.949  1.00 25.42      C3
ATOM  3757 CG  ARG  548      61.820  39.361   0.294  1.00 26.11      C3
ATOM  3758 CD  ARG  548      63.280  39.158  -0.054  1.00 29.84      C3
ATOM  3759 NE  ARG  548      64.044  39.162   1.189  1.00 32.30      C3
ATOM  3760 HE  ARG  548      63.572  38.883   1.995  1.00  0.00      C3
ATOM  3761 CZ  ARG  548      65.344  39.518   1.225  1.00 32.66      C3
ATOM  3762 NH1 ARG  548      66.159  39.923   0.255  1.00 34.98      C3
ATOM  3763 HH11 ARG  548      67.107  40.170   0.533  1.00  0.00      C3
ATOM  3764 HH12 ARG  548      65.812  39.981  -0.600  1.00  0.00      C3
ATOM  3765 NH2 ARG  548      65.837  39.518   2.549  1.00 32.03      C3
ATOM  3766 HH21 ARG  548      66.788  39.761   2.708  1.00  0.00      C3
ATOM  3767 HH22 ARG  548      65.250  39.275   3.321  1.00  0.00      C3
ATOM  3768 C   ARG  548      58.713  38.997  -1.832  1.00 29.81      C3
ATOM  3769 O   ARG  548      57.778  39.790  -1.968  1.00 34.54      C3
ATOM  3770 N   ALA  549      58.979  38.102  -2.761  1.00 27.87      C3
ATOM  3771 H   ALA  549      59.684  37.436  -2.601  1.00  0.00      C3
ATOM  3772 CA  ALA  549      58.227  38.045  -3.984  1.00 27.18      C3
```

FIGURE 5

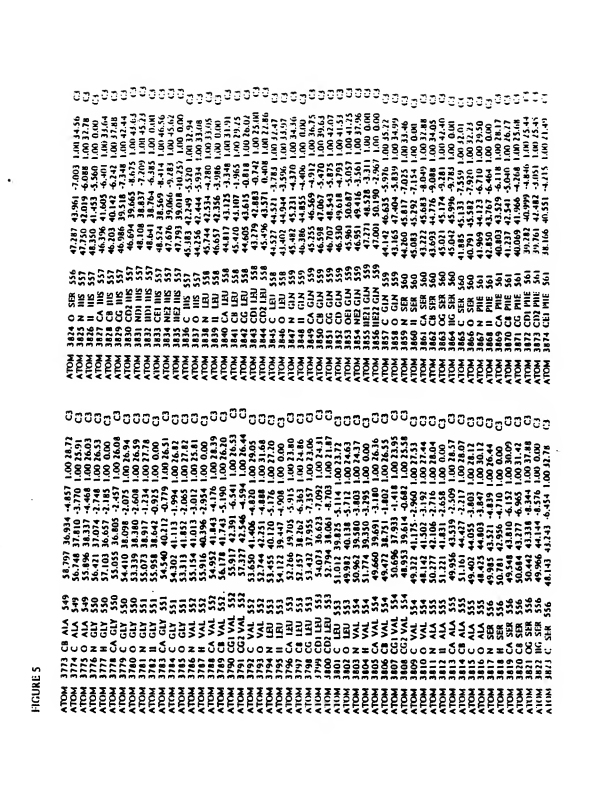

FIGURE 5

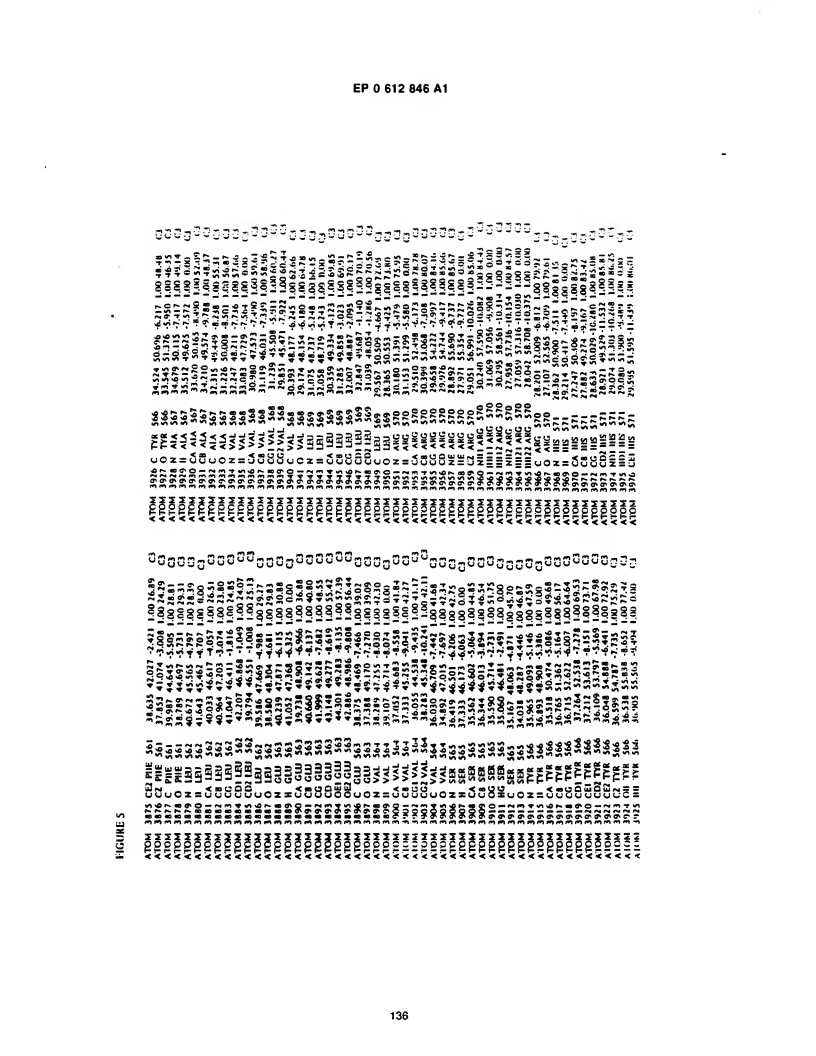

FIGURE 5

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ATOM  3977 NE2 HIS  571      29.494  50.518 -12.187  1.00 86.28      C3
ATOM  3978 HE2 HIS  571      29.801  50.468 -13.119  1.00  0.00      C3
ATOM  3979 C   HIS  571      26.225  49.759  -7.195  1.00 83.31      C3
ATOM  3980 O   HIS  571      25.075  50.194  -7.301  1.00 84.06      C3
ATOM  3981 N   LEU  572      26.540  48.963  -6.158  1.00 83.11      C3
ATOM  3982 H   LEU  572      27.474  48.874  -5.915  1.00  0.00      C3
ATOM  3983 CA  LEU  572      25.527  48.457  -5.241  1.00 83.71      C3
ATOM  3984 CB  LEU  572      26.085  47.267  -4.454  1.00 83.57      C3
ATOM  3985 CG  LEU  572      25.439  45.884  -4.721  1.00 83.79      C3
ATOM  3986 CD1 LEU  572      25.783  45.346  -6.127  1.00 84.16      C3
ATOM  3987 CD2 LEU  572      25.958  44.866  -3.714  1.00 84.08      C3
ATOM  3988 C   LEU  572      24.997  49.511  -4.261  1.00 84.78      C3
ATOM  3989 O   LEU  572      24.265  49.192  -3.295  1.00 84.85      C3
ATOM  3990 N   ALA  573      25.349  50.796  -4.483  1.00 85.56      C3
ATOM  3991 H   ALA  573      26.020  50.980  -5.174  1.00  0.00      C3
ATOM  3992 CA  ALA  573      24.822  51.925  -3.721  1.00 85.90      C3
ATOM  3993 CB  ALA  573      25.600  53.207  -3.970  1.00 85.79      C3
ATOM  3994 C   ALA  573      23.373  52.245  -4.057  1.00 87.21      C3
ATOM  3995 OT1 ALA  573      22.610  52.413  -3.099  1.00 88.33      C3
ATOM  3996 OT2 ALA  573      23.022  52.298  -5.248  1.00 88.34      C3
ATOM  3997 OH2 H2O  603      26.735  24.280   5.161  1.00 27.42      W
ATOM  3998 H1  H2O  603      27.332  24.335   4.407  1.00  0.00      W
ATOM  3999 H2  H2O  603      26.188  23.435   4.992  1.00  0.00      W
ATOM  4000 OH2 H2O  605      47.880  37.960  12.073  1.00 56.30      W
ATOM  4001 H1  H2O  605      47.789  37.874  13.031  1.00  0.00      W
ATOM  4002 H2  H2O  605      46.980  37.858  11.753  1.00  0.00      W
ATOM  4003 OH2 H2O  607      40.001  49.224   7.214  1.00 40.04      W
ATOM  4004 H1  H2O  607      40.471  48.761   7.909  1.00  0.00      W
ATOM  4005 H2  H2O  607      40.123  48.647   6.457  1.00  0.00      W
ATOM  4006 OH2 H2O  610      59.883  42.530  -9.698  1.00 38.90      W
ATOM  4007 H1  H2O  610      60.512  41.833  -9.477  1.00  0.00      W
ATOM  4008 H2  H2O  610      59.189  42.606 -10.360  1.00  0.00      W
ATOM  4009 OH2 H2O  611      57.178  35.940 -14.220  1.00 34.63      W
ATOM  4010 H1  H2O  611      57.174  36.545 -14.974  1.00  0.00      W
ATOM  4011 H2  H2O  611      57.989  36.211 -13.757  1.00  0.00      W
ATOM  4012 OH2 H2O  612      25.793  27.337  19.130  1.00 29.21      W
ATOM  4013 H1  H2O  612      26.709  27.661  19.145  1.00  0.00      W
ATOM  4014 H2  H2O  612      25.762  26.792  19.929  1.00  0.00      W
ATOM  4015 OH2 H2O  615      29.766  34.284   9.444  1.00 45.03      W
ATOM  4016 H1  H2O  615      30.017  34.618  10.308  1.00  0.00      W
ATOM  4017 H2  H2O  615      29.113  33.592   9.660  1.00  0.00      W
ATOM  4018 OH2 H2O  617      37.316  40.012  10.872  1.00 35.21      W
ATOM  4019 H1  H2O  617      36.600  40.017  11.519  1.00  0.00      W
ATOM  4020 H2  H2O  617      37.944  39.376  11.259  1.00  0.00      W
ATOM  4021 OH2 H2O  619      40.370  52.041  -7.387  1.00 29.62      W
ATOM  4022 H1  H2O  619      40.672  52.724  -6.779  1.00  0.00      W
ATOM  4023 H2  H2O  619      39.505  51.810  -7.052  1.00  0.00      W
ATOM  4024 OH2 H2O  621      37.903  32.440  10.664  1.00 39.99      W
ATOM  4025 H1  H2O  621      27.553  33.207  11.141  1.00  0.00      W
ATOM  4026 H2  H2O  621      27.929  31.808  11.398  1.00  0.00      W
ATOM  4027 OH2 H2O  622      25.057  31.972  13.675  1.00 42.70      W
ATOM  4028 H1  H2O  622      24.393  32.417  14.215  1.00  0.00      W
ATOM  4029 H2  H2O  622      24.469  31.428  13.112  1.00  0.00      W
ATOM  4030 OH2 H2O  623      20.791  28.583  14.218  1.00 50.17      W
ATOM  4031 H1  H2O  623      20.499  28.803  13.325  1.00  0.00      W
ATOM  4032 H2  H2O  623      19.939  28.549  14.688  1.00  0.00      W
ATOM  4033 OH2 H2O  625      22.680  78.881   2.761  1.00 40.48      W
ATOM  4034 H1  H2O  625      21.938  78.856   3.375  1.00  0.00      W
ATOM  4035 H2  H2O  625      22.266  79.246   1.970  1.00  0.00      W
ATOM  4036 OH2 H2O  626      39.689  36.486   9.740  1.00 23.36      W
ATOM  4037 H1  H2O  626      39.090  35.724   9.677  1.00  0.00      W
ATOM  4038 H2  H2O  626      39.627  36.872   8.853  1.00  0.00      W
ATOM  4039 OH2 H2O  627      42.035  78.320   5.697  1.00 46.19      W
ATOM  4040 H1  H2O  627      42.416  77.450   5.832  1.00  0.00      W
ATOM  4041 H2  H2O  627      41.243  78.146   5.181  1.00  0.00      W
ATOM  4042 OH2 H2O  631      47.227  31.440   6.299  1.00 34.17      W
ATOM  4043 H1  H2O  631      47.533  32.209   5.809  1.00  0.00      W
ATOM  4044 H2  H2O  631      47.442  30.713   5.714  1.00  0.00      W
ATOM  4045 OH2 H2O  636      24.043  65.423  -0.336  1.00 73.18      W
ATOM  4046 H1  H2O  636      24.179  65.781  -1.228  1.00  0.00      W
ATOM  4047 H2  H2O  636      23.469  66.096   0.054  1.00  0.00      W
ATOM  4048 OH2 H2O  638      38.984  67.955 -11.226  1.00 29.97      W
ATOM  4049 H1  H2O  638      38.283  67.402 -11.580  1.00  0.00      W
ATOM  4050 H2  H2O  638      38.548  68.664 -11.698  1.00  0.00      W
ATOM  4051 OH2 H2O  639      27.930  66.675  -7.733  1.00 43.40      W
ATOM  4052 H1  H2O  639      28.192  67.028  -6.876  1.00  0.00      W
ATOM  4053 H2  H2O  639      26.982  66.791  -7.705  1.00  0.00      W
ATOM  4054 OH2 H2O  643      50.619  62.802   0.811  1.00 36.55      W
ATOM  4055 H1  H2O  643      51.575  62.904   0.824  1.00  0.00      W
ATOM  4056 H2  H2O  643      50.301  63.665   0.525  1.00  0.00      W
ATOM  4057 OH2 H2O  646      62.897  38.367   3.759  1.00 73.55      W
ATOM  4058 H1  H2O  646      62.414  38.098   2.978  1.00  0.00      W
ATOM  4059 H2  H2O  646      62.246  38.247   4.463  1.00  0.00      W
ATOM  4060 OH2 H2O  650      29.587  68.480  -9.555  1.00 65.67      W
ATOM  4061 H1  H2O  650      28.846  68.630 -10.148  1.00  0.00      W
ATOM  4062 H2  H2O  650      29.180  67.844  -8.936  1.00  0.00      W
ATOM  4063 OH2 H2O  652      51.408  56.331   4.056  1.00 62.40      W
ATOM  4064 H1  H2O  652      50.718  56.353   3.365  1.00  0.00      W
ATOM  4065 H2  H2O  652      51.052  55.671   4.648  1.00  0.00      W
ATOM  4066 OH2 H2O  653      49.404  56.022   2.161  1.00 51.28      W
ATOM  4067 H1  H2O  653      49.442  55.351   1.474  1.00  0.00      W
ATOM  4068 H2  H2O  653      49.323  56.829   1.630  1.00  0.00      W
ATOM  4069 OH2 H2O  654      68.215  42.294  -2.563  1.00 40.77      W
ATOM  4070 H1  H2O  654      68.347  41.745  -1.777  1.00  0.00      W
ATOM  4071 H2  H2O  654      68.189  43.181  -2.190  1.00  0.00      W
ATOM  4072 OH2 H2O  655      66.374  40.425  -2.489  1.00 47.31      W
ATOM  4073 H1  H2O  655      66.936  41.162  -2.766  1.00  0.00      W
ATOM  4074 H2  H2O  655      66.452  39.841  -3.252  1.00  0.00      W
ATOM  4075 OH2 H2O  656      66.927  41.428  -5.011  1.00 44.08      W
ATOM  4076 H1  H2O  656      66.207  42.071  -4.989  1.00  0.00      W
ATOM  4077 H2  H2O  656      67.542  41.824  -4.474  1.00  0.00      W
ATOM  4078 OH2 H2O  657      40.371  57.111   5.230  1.00 46.55      W
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FIGURE 5

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ATOM  4079 H1  H2O  657     39.958 56.259  5.613  1.00  0.00      W
ATOM  4080 H2  H2O  657     40.021 57.651  5.014  1.00  0.00      W
ATOM  4081 OH2 H2O  658     48.780 47.580 -3.122  1.00 52.09      W
ATOM  4082 H1  H2O  658     48.811 46.671 -3.438  1.00  0.00      W
ATOM  4083 H2  H2O  658     49.568 47.955 -3.542  1.00  0.00      W
ATOM  4084 OH2 H2O  663     29.095 62.889  1.825  1.00 39.23      W
ATOM  4085 H1  H2O  663     29.380 62.827  2.739  1.00  0.00      W
ATOM  4086 H2  H2O  663     28.377 63.526  1.887  1.00  0.00      W
ATOM  4087 OH2 H2O  664     27.132 25.640  7.430  1.00 50.65      W
ATOM  4088 H1  H2O  664     26.870 24.838  7.876  1.00  0.00      W
ATOM  4089 H2  H2O  664     27.001 25.362  6.496  1.00  0.00      W
ATOM  4090 OH2 H2O  665     23.367 30.554 12.167  1.00 49.69      W
ATOM  4091 H1  H2O  665     24.026 30.006 11.707  1.00  0.00      W
ATOM  4092 H2  H2O  665     22.941 31.016 11.438  1.00  0.00      W
ATOM  4093 OH2 H2O  666     46.015 32.192 10.179  1.00 66.86      W
ATOM  4094 H1  H2O  666     46.060 31.519  9.497  1.00  0.00      W
ATOM  4095 H2  H2O  666     45.411 31.827 10.833  1.00  0.00      W
ATOM  4096 OH2 H2O  667     38.943 37.883 11.978  1.00 47.87      W
ATOM  4097 H1  H2O  667     39.367 37.487 11.188  1.00  0.00      W
ATOM  4098 H2  H2O  667     38.521 37.114 12.362  1.00  0.00      W
ATOM  4099 OH2 H2O  671     33.437 58.101  2.269  1.00 46.65      W
ATOM  4100 H1  H2O  671     33.555 57.162  2.433  1.00  0.00      W
ATOM  4101 H2  H2O  671     33.962 58.514  2.961  1.00  0.00      W
ATOM  4102 OH2 H2O  672     27.551 31.314 20.022  1.00 30.15      W
ATOM  4103 H1  H2O  672     27.929 32.042 20.533  1.00  0.00      W
ATOM  4104 H2  H2O  672     26.845 31.764 19.552  1.00  0.00      W
ATOM  4105 OH2 H2O  673     25.714 36.908 21.385  1.00 36.95      W
ATOM  4106 H1  H2O  673     24.806 37.123 21.637  1.00  0.00      W
ATOM  4107 H2  H2O  673     25.599 36.284 20.654  1.00  0.00      W
ATOM  4108 OH2 H2O  674     38.244 66.897 12.076  1.00 57.36      W
ATOM  4109 H1  H2O  674     37.773 67.536 12.626  1.00  0.00      W
ATOM  4110 H2  H2O  674     38.153 66.104 12.618  1.00  0.00      W
ATOM  4111 OH2 H2O  675     35.762 36.553 -3.986  1.00 58.40      W
ATOM  4112 H1  H2O  675     35.600 37.449 -3.677  1.00  0.00      W
ATOM  4113 H2  H2O  675     35.549 36.642 -4.923  1.00  0.00      W
ATOM  4114 OH2 H2O  676     30.689 32.814 25.675  1.00 59.30      W
ATOM  4115 H1  H2O  676     30.093 33.571 25.680  1.00  0.00      W
ATOM  4116 H2  H2O  676     31.550 33.214 25.540  1.00  0.00      W
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```


EP 0 612 846 A1

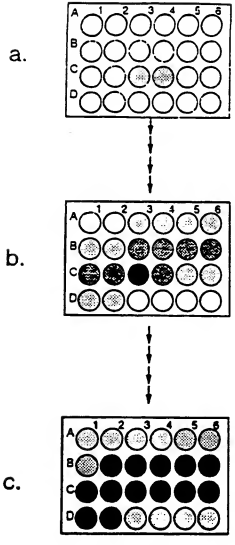

FIGURE 6

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 94 10 1207

DOCUMENTS CONSIDERED TO BE RELEVANT

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X D	WO-A-89 05824 (GENETICS INSTITUTE, INC.) * the whole document especially page 17 table 2 , page 21 lines 16-19 and page 22 lines 25-37 * & US-A-4 904 584 (GENETICS INSTITUTE) --- -/--	17-22	

TECHNICAL FIELDS SEARCHED (Int.Cl.5)
C12N
C07K
C12P

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	11 May 1994	Le Cornec, N

CATEGORY OF CITED DOCUMENTS

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